ENVIRONMENT ONTARIO RESEARCH REPORT

HUMBER RIVER BACTERIOLOGICAL STUDY

prepared for

Ontario Ministry of the Environment

RAC Project No. 113 PL

by

University of Toronto
Department of Microbiology

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Identification of Faecal Coliforms and Faecal Streptococci and Verification of Newer Tests to Determine Human and Non-Human Faeces

RAC Project No. 113 PL

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DISCLAIMER

This project report has been prepared for the Water Resources Branch and the Research Advisory Committee in fulfillment of the terms of the funding. The views expressed are those of the authors and do not necessarily reflect the views and policies of the Ontario Ministry of the Environment.

ABSTRACT

A bacteriological study of the Humber River Basin within the Metropolitan Toronto boundaries was conducted during the fall of 1983 (Gore and Storrie), to assess the bacteriological water quality of the Humber River and Black Creek and to identify the origin of fecal contamination in both watercourses (i.e. human or nonhuman). Two newly developed media for the isolation and enumeration of E. coli (m-TEC-IG with indoxyl-β-D-glucoside) and enterococci (m-ME with indoxyl-β-D-glucoside) (Dufour 1979, 1980) were tested under field conditions during the study.

The specificity of the new media were assessed using data pertaining to the isolation and identification of 3683 bacterial isolates taken from samples analyzed during the study. Overall, the m-TEC-IG medium provided a good estimation of the concentration of E. coli. There was, however, a tendency for a slight overestimation especially during wet weather conditions. The m-ME medium appeared to be acceptable for the enumeration of enterococci during both wet and dry weather events, but gave a slight underestimation of the actual concentration of enterococci.

Information on the bacterial populations in the Humber River and Black Creek was studied to determine the original sources of faecal contamination. The data yielded only a limited number of conclusions due to the fact that only six sampling stations along the entire river course were assessed (four during wet weather)

A predominance of S. faecium in both Black Creek and the Humber River during dry weather, especially at the downstream stations, indicated possible increases in human inputs to both watercourses in a downstream direction. As well, a greater impact of human faecal wastes was seen in Black Creek as opposed to the Humber River. Increased recovery of S. faecium var. casseliflavis at station H3 may reflect inputs from the bird population in that area.

The wet weather recoveries indicated that human faecal inputs may be higher during wet weather compared to dry weather results; however, their impact was masked by an increase in non-human faecal and non-faecal inputs as well as less recent inputs, possibly caused by sediment resuspension. The data collected from

the second wet event suggested greater human faecal input than during the first wet event possibly due to increased flows and the greater duration of the event. Again, Black Creek exhibited a greater human faecal input than did the Humber River.

Overall, the approach to evaluating potential sources of faecal material through bacterial identifications has definite value but would be more productive if used in conjunction with studies of single inputs and a more selective sampling program.

RÉSUMÉ

À l'automne de 1983, on a effectué une étude bactériologique dans le bassin de la rivière Humber (Gore et Storrie), plus précisément dans les limites de la communauté urbaine de Toronto, pour évaluer la qualité bactériologique de l'eau de la rivière Humber et du ruisseau Black et cerner l'origine (humaine ou animale) de la contamination fécale dans les deux cours d'eau. Deux nouveaux milieux servant à isoler et à dénombrer les E. coli (m-TEC-IG avec de l'indoxyle-B-D-glucoside) et les entérocoques (m-Me avec de l'indoxyle-B-D-glucoside) (Dufour 1979, 1980) ont été mis à l'essai sur le terrain pendant l'étude.

On a évalué la spécificité des nouveaux milieux à l'aide de données sur l'isolation et l'identification de 3 683 bactéries isolées provenant d'échantillons analysés durant l'étude. De façon générale, le milieu m-TEG-IG a permis d'obtenir une bonne estimation de la concentration d'E. coli. Toutefois, il s'est dégagé une légère tendance à la surestimation, surtout par temps humide ou pluvieux. Quant au milieu m-ME, il semblait permettre un dénombrement acceptable des entérocoques, par temps sec et humide ou pluvieux, mais il a donné une concentration réelle légèrement sous-estimée des entérocoques.

On a étudié les renseignements existants sur les populations bactériennes dans la rivière Humber et le ruisseau Black en vue de repérer les sources premières de contamination fécale. Les données analysées n'ont permis de tirer qu'un nombre limité de conclusions, étant donné que seulement six stations de prélèvement ont fait l'objet d'une évaluation sur toute la longueur de la rivière (quatre par temps pluvieux).

La prédominance de S. Faecium dans le ruisseau Black et aussi dans la rivière Humber par temps sec, notamment à la hauteur des stations d'aval, indiquait une augmentation possible de l'apport en excréments humains dans les deux cours d'eau vers l'aval. En outre, les effets imputables aux matières fécales humaines étaient plus considérables dans le ruisseau Black que dans la rivière Humber. Les excréments d'oiseaux déposés dans la région de la station H3 expliquent sans doute les quantités accrues de S. Faecium var. casseliflavis y ayant été prélevées.

Les prélèvements effectués par temps pluvieux ont porté à conclure que les apports en matières humaines sont peut-être plus importants par temps humide que par temps sec. Toutefois, leurs effets ont été masqués par des apports accrus de matières fécales et non fécales autres qu'humaines et par des apports moins récents probablement provoqués par la remise en suspension de sédiments. Les données correspondant au deuxième prélèvement effectué par temps humide ou pluvieux ont laissé supposer que l'apport en matières fécales humaines était plus grand qu'au premier prélèvement, en raison peut-être du débit plus fort et de la plus grande durée du second épisode pluvieux. Cette fois encore, la concentration de matières fécales humaines était plus forte dans le ruisseau Black que dans la rivière Humber.

En termes généraux, l'évaluation des sources potentielles de matières fécales au moyen de l'identification de bactéries est d'une utilité certaine, mais cette méthode donnerait de meilleurs résultats si l'on y adjoignait des études sur les apports isolés et un programme d'échantillonnage plus sélectif.

Humber River Bacteriological Study
Identification of Faecal Coliforms and Faecal Stretococci and
Verification of Newer Tests to Determine Human
and Non-Human Faeces

T.A.W.M.S./R.A.C. Technical Report

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Introduction

Bacteriological monitoring of the Humber River and Black Creek have shown that both watercourses have high concentrations of faecal indicator bacteria suggesting heavy faecal loading. In order to better define the extent and nature of existing faecal pollution problems, a bacteriological study of the Humber River Basin within the Metropolitan Toronto boundaries was conducted during the fall of 1983 (Gore and Storie 1984). The survey was undertaken as part of the Toronto Area Watershed Management Study (T.A.W.M.S.) to:

a) assess the bacteriological water quality of both the Humber River and Black Creek; and

b) to identify the origins of faecal contamination in the Humber River and in Black Creek.

This and previous studies (T.A.W.M.S., Fall 1982 and July 1983) reported that the bacteriological quality in the Humber River and Black Creek does not comply with the PWQO and IJC recommended objectives (Gore and Storie 1984). Levels of faecal indicator bacteria at many locations approach levels indicative of heavy inputs of human and animal waste. Contributing to this heavy loading is faecal material washed into the river from park and pasture-land runoff (domestic and wild animals), combined sewers, storm sewers, and bird roosting areas (e.g. bridges and islands). Pinpointing the original source of the faecal pollution is difficult due to the use of interpretive tools based on older methods, the limited number of sampling sites involved, and the paucity of current information on the bacteriology of faeces.

Media and methods currently used by the Ministry of the Environment to isolate and enumerate faecal coliforms and faecal streptococci are different from those used by Geldreich in 1969 when he first proposed the use of faecal coliform to faecal streptococcus ratios (FC/FS). Furthermore the limited number of sampling sites chosen for monitoring may further alter the significance of the FC/FS ratios because, according to Geldreich (1969), ratios become invalid downstream from the source. Monitoring downstream changes in FC/FS ratios (Feachem 1974) may be more valuable as a means of detecting sources, but would not be applicable where

input sites, contributing faeces from different sources, are in close proximity to one another.

The validity of using Geldreich's FC/FS ratios with current methods is now questioned because of the results of a study of the bacteriology of faeces being conducted at the University of Toronto (P. Seyfried, E. Harris and M. Young, in progress). These results show that some major species of animals in the Toronto area can have FC/FS ratios similar to humans. This is possibly due to ingestion of the same types of foods, or to close association with humans or waters that are polluted with human wastes.

Past studies on the bacteriology of faeces have shown that the differences between humans and animals may be more readily detected through the types of bacteria carried than through the levels of bacteria and ratios obtained (Kenner 1978, Mundt 1982 and Mead 1972). Similar results are being found by the University of Toronto Faecal Study. It is anticipated that determination of the relative densities of various species of faecal streptococci in surface waters may provide information on the original source of faecal pollution.

One significant fact brought to light by the past and recent studies on the bacteriology of faeces is that both humans and animals carry E. coli and enterococci (a subgroup of faecal streptococci comprised of S. faecalis, S. faecium and S. durans) and that these bacteria do not multiply outside the host, nor are they found elswhere in the environment+ (Mundt 1982 and Phirke and Verma 1972). It has been proposed, based on this information, that E. coli and enterococci be used as faecal pollution indicators because they are better related to levels of faecal contamination than faecal coliforms and faecal streptococci. Media for the isolation and enumeration of these bacteria have been recently developed in the United States (Dufour 1970, 1980) but have not been fully tested in Ontario.

Based on the data obtained to date, the objectives of this study were two-fold:

1) To assess the specificity under field conditions of the media for the isolation and enumeration of E. coli (M-TEC with indoxl-^{R}-D-glucoside: m-TEC-IG) and enterococci (m-E with indoxyl-β-D-glucoside: m-ME) developed by Dufour.

2) To provide additional information for the identification of the original sources of faecal pollution (i.e. human or non-human).

Both objectives required the isolation and identification of bacteria from media used in the Humber River/Black Creek survey (Gore and Storrie 1984). In the assessment of results, the identity of bacteria isolated from m-TEC-IG and m-ME, would determine how well the target colony count reported for each media related to the actual concentration of target organisms. Also the genera and species of bacteria isolated from the river and creek would be compared with data on the bacterial populations of faeces to assist in determining potential sources of pollution.

+ Some studies (Mundt 1982) indicate that certain subspecies of S. faecalis and S. faecium may be found on plants and insects.

Study Components

The various components of this study are as follows:

1) Isolation and preservation of 3683 bacterial isolates from the Humber River/Black Creek bacteriological study (Gore and Storrie 1984).

2) Complete biochemical and, where necessary, serological identification of all isolates collected.

3) Assessment of two new isolation media for the recovery of E. coli and enterococci based upon bacterial identification.

4) Interpretation of bacterial identification for each sampling station for wet and dry weather conditions.

5) Determination of the potential origin of faecal pollution by identification of indicator bacteria.

Methods

Samples were collected from 4 sites along the Humber River and 2 sites along Black Creek (Figures 1 and 2) Information relating to the sampling and bacteriological analysis to assess water quality are detailed in the Humber River/Black Creek report (Gore and Storrie, 1984). A total of 3638 bacterial isolates were picked from 2 routine faecal indicator media; m-TEC (faecal coliforms) and m-Enterococcus (faecal streptococci) and 2 test media; m-TEC-IG (E. coli) and m-ME (enterococci). The formulations and specification for all 4 isolation media are listed in Appendix A.

Isolates were selected from one filter containing colonies within the acceptable counting range of 10-150 target colonies for each of the 4 media. Ten percent or a minimum of 10 colonies were picked from one representative area of the selected filter. The colonies were streaked onto nutrient agar plates and incubated overnight to check the purity of the isolate. Pure isolates were transferred to and stored on brain-heart infusion agar slants under sterile parafin oil in a 20°C humidity controlled storage room until they could be identified.

Before identification, the isolates were subcultured onto nutrient agar plates and incubated overnight to obtain fresh growth and to recheck purity. Isolates from m-TEC and m-TEC-IG agar were tested for their Gram reaction using a 3% potassium hydroxide solution (Fluharty and Packard 1967). All Gram negative isolates were identified using API 20E biochemical test strips for the identification of Enterobacteriaceae.

Pure isolates taken from m-Enterococcus (m-ENT) and m-ME agar were tested for Gram (3% KOH) and catalase reactions (3% hydrogen peroxide solution). All Gram positive, catalase negative isolates were identified according to an identification scheme developed at the Ministry of the Environment (Personal communication, G. Horsenell). The method incorporated both biochemical tests and seriological tests to determine the presence of the group D antigen. Details of the method are listed in Appendix B along with the references used to develop the methods.

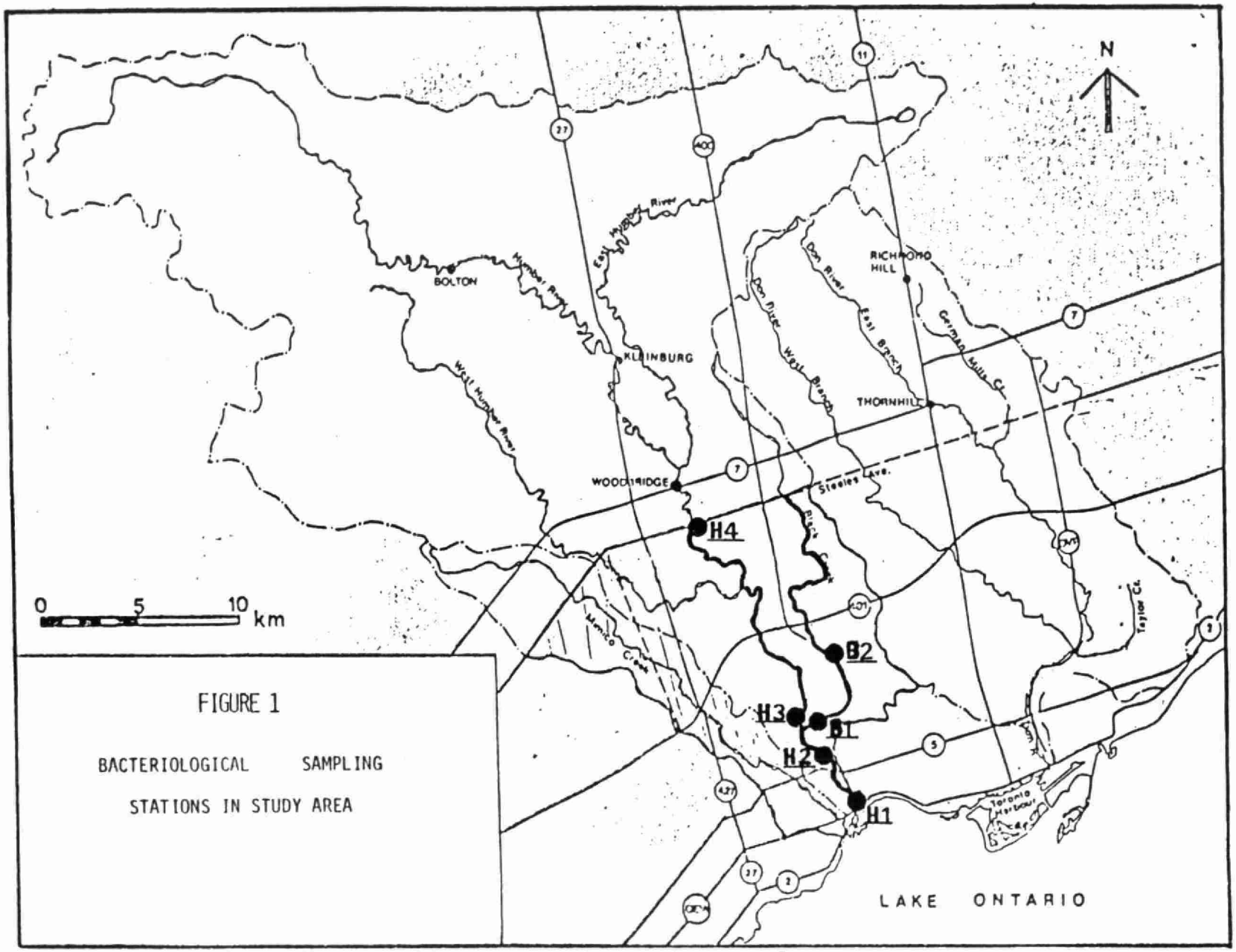
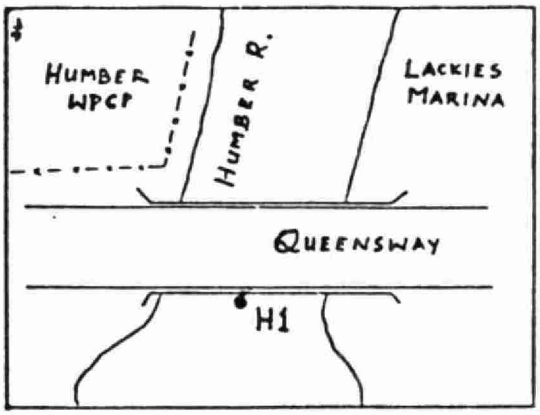
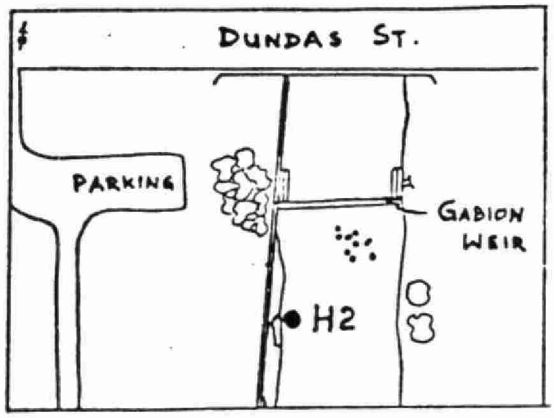


FIGURE 1

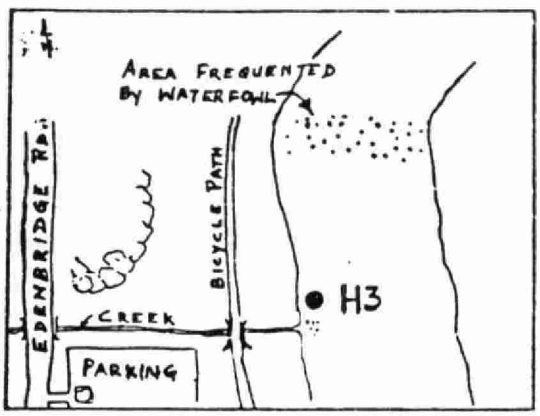
BACTERIOLOGICAL SAMPLING STATIONS IN STUDY AREA



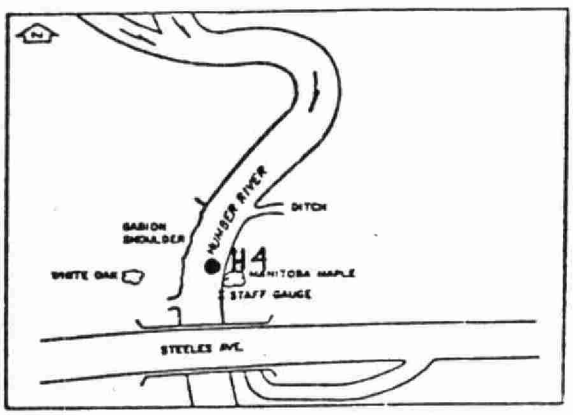
(a) Humber R. @ Lakeshore Blvd.



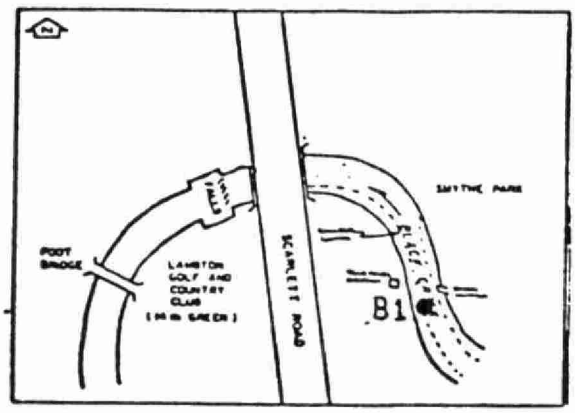
(b) Humber R. @ Dundas St.



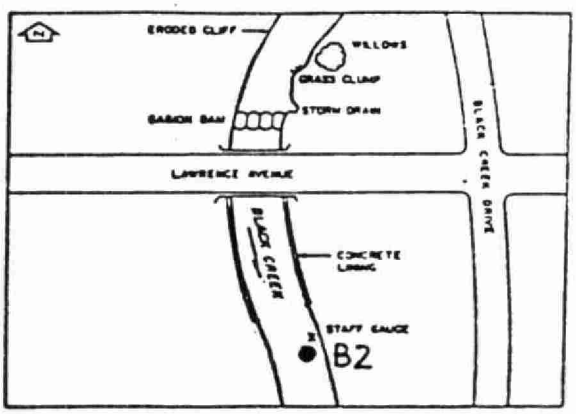
(c) Humber R. @ James Gardens



(d) Humber R. @ Steeles Ave.



(e) Black Cr. @ Scarlett Rd.



(f) Black Cr. @ Lawrence Ave.

FIGURE 2 DETAILED SITE MAPS

Results

Summary of Media Recovery During Wet and Dry Weather Surveys

An overall summary of the bacteria isolated and identified under dry and wet weather conditions for each of the four selective media (m-TEC, m-TEC-IG, m-Ent and m-ME) is provided in Tables 1 and 2.

A high percentage of target colonies on both m-TEC and m-TEC-IG (Table 1) were Enterobacteriaceae, but, the distribution of the genera isolated differed. The m-TEC-IG medium recovered a higher percentage of E. coli as targets than m-TEC while m-TEC recovered a higher level of Klebsiella spp. and Enterobacter spp. as targets. These non-E. coli Enterobacteriaceae make up a higher percentage of non-target colonies on m-TEC-IG than on m-TEC.

Wet weather survey conditions resulted in a drop in the percent E. coli recovered as target colonies on both media, although this was much more noticeable on m-TEC.

A high percentage of target colonies identified on m-ME and m-Ent agar (Tabel 2) were faecal streptococci, with the majority of these being enterococci. The m-ME had a somewhat higher percentage recovery of enterococci targets than m-Ent and a higher non-target colony recovery, however, close to 60 percent of these non-targets were enterococci as well.

There were noticeable differences in the streptococcal species isolated on each medium. For example, the m-Ent agar recovered a higher percentage of S. avium and non-faecal streptococci as target colonies, while on m-ME, S. bovis was isolated more frequently from target colonies. Both S. faecium and S. faecium var. casseliflavus constituted a different percentage of target colonies depending upon the weather conditions, but in all cases they made up a significant part of the streptococcal population on both media.

TABLE 1: Humber River/Black Creek Study: Media Summary for Wet and Dry Weather Conditions Using m-TEC and m-TEC-IG Agar

	Dry Weather								Wet Weather							
	m-TEC				m-TEC-IG				m-TEC				m-TEC-IG			
	TAR[a]	ID(%)	Bkgd[b]	ID(%)	TAR	ID(%)	Bkgd	ID(%)	TAR	ID(%)	Bkdg	ID(%)	TAR	ID(%)	Bkgd	ID(%)
Total (all stations)	374(100)		89(100)		305(100)		161(100)		375(100)		129(100)		319(100)		188(100)	
Entero-bacteriaceae	367	(98.1)	62	(69.7)	302	(99.0)	141	(87.6)	361	(96.3)	95	(73.6)	314	(98.4)	167	(88.8)
E. coli	241	(64.4)	14	(15.7)	267	(87.5)	20	(12.4)	211	(56.3)	12	(9.1)	269	(84.3)	26	(13.8)
Klebsiella spp.	72	(19.3)	16	(18.0)	12	(3.9)	83	(51.6)	73	(19.5)	17	(12.9)	16	(5.0)	89	(47.3)
Enterobacter spp.	36	(9.6)	25	(28.1)	7	(2.3)	31	(19.3)	46	(12.3)	49	(37.1)	13	(4.1)	42	(22.3)
Citrobacter spp.	14	(3.7)	4	(4.5)	11	(3.6)	5	(3.1)	28	(7.5)	17	(12.9)	13	(4.1)	6	(3.2)
Other	4	(1.1)	3	(3.4)	5	(1.6)	2	(1.2)	1	(0.3)	-	-	3	(0.9)	4	(2.1)
Citrobacter spp. or E. coli	-		-		-		-		2	(0.5)	-		-		-	-
Non-Entero-bacteriaceae	4	(1.1)	22	(24.7)	2	(0.7)	16	(9.9)	4	(1.1)	32	(24.8)	2	(0.6)	17	(9.0)
G.L.L.S.[c]	3	(0.8)	5	(5.6)	1	(0.3)	4	(2.5)	10	(2.7)	2	(1.6)	3	(0.9)	4	(2.1)

[a]TAR ID = Target identification
[b]Bkgd = Background
[c]G.L.L.S. = Good likelihood low selectivity

TABLE 2: Humber River/Black Creek Study: Media Summary for Wet and Dry Weather Conditions Using m-ENT and m-ME Agar

	Dry Weather								Wet Weather							
	m-ENT				m-ME				m-ENT				m-ME			
	TAR[a]	ID(%)	Bkgd	ID(%)	TAR	ID(%)	Bkgd	ID(%)	TAR	ID(%)	Bkdg	ID(%)	TAR	ID(%)	Bkgd	ID(%)
Total (all stations)	434		18		321		71		471		0		336		97	
Enterococci	377	(86.9)	4	(22.2)	302	(94.1)	48	(67.7)	374	(79.4)			300	(89.3)	57	(58.8)
S. faecalis	3	(0.7)	–		3	(1.0)	1	(1.4)	7	(1.5)			2	(0.6)	1	(1.0)
Var Liquefaciens	38	(8.8)	–		43	(13.4)	1	(1.4)	76	(16.1)			43	(12.8)	–	
Var faecalis	18	(4.2)	1	(5.6)	18	(5.6)	_		46	(9.8)			21	(6.2)	5	(5.2)
Var zymogenes	5	(1.2)	-		2	(0.6)	-		1	(0.2)			3	(0.9)	_	
S. faecium	133	(30.6)	1	(5.6)	140	(43.6)	27	(38.0)	94	(20.0)			118	(35.0)	40	(41.2)
Var casseliflavus	115	(26.5)	2	(11.1)	41	(12.8)	11	(15.5)	73	(15.5)			71	(21.1)	7	(7.2)
S. durans	65	(15.0)			55	(17.1)	8	(11.3)	77	(16.3)			42	(12.5)	4	(4.1)
Faecal Streptococci, Non-Enterococci	15	(3.5)			8	(2.5)	–		26	(5.5)			20	(5.9)	2	(2.1)
S. bovis	–				6	(1.9)	_		6	(1.3)			16	(4.8)	–	
S. bovis (Var)	–				1	(0.3)	–		–				1	(0.6)	_	
S. avium	15	(3.5)			1	(0.3)	_		20	(4.3)			3	(0.9)	2	(2.1)
S. equinus	-		-		-		-		-		-		-		-	
Non-Faecal Strep	42	(9.6)	14	(77.8)	11	(3.4)	23	(32.4)	71	(51.1)			16	(4.8)	38	(39.2)

Summary of Bacterial Identification by Station and Survey

A summary of target colony identification by station and survey (dry weather, wet weather 1 and 2) is provided in Tables 3 to 14.

Faecal Coliforms (m-TEC) Dry Weather (Table 3)

There was a lower percent recovery (%R) of E. coli on the two downstream stations on the Humber River (H1 and H2) than on the stations further upstream (H3 and H4); however, the actual concentration showed a steady increase from H4 to H1). The %R's of E. coli from Black Creek were similar to those at H1 and H2, with B2 having a higher concentration than B1 and both H2 and H1 having higher concentrations than B2. The stations with lower %R's of E. coli had higher Klebsiella spp. recoveries, particularly station B1. The most heterogeneous faecal coliform population was recovered at B2. Concentrations of E. coli recovered decreased as follows: B1 B2 H1 H2 H3 H4.

Escherichia coli (m-TEC-IG) Dry Weather (Table 4)

The %R's of E. coli were higher from mTEC-IG than from m-TEC and there was less variation from station to station. Actual concentrations of E. coli determined on this medium also decreased in a downstream direction and were in good agreement with the levels detected by m-TEC but were not reported as part of the Humber River/Black Creek study. The percentage Klebsiella spp. was again highest at B1, and B2 had the most heterogeneous faecal coliform population.

TABLE 3:
Humber River/Black Creek Study: Dry Weather Results by Station m-TEC Agar

	H1		H2		H3		H4		B1		B2	
	Tar	ID(%) Tar Conc	Tar	ID(%) Tar Conc	Tar	ID (%) Tar Conc	Tar	ID(%) Tar Conc	Tar	ID(%) Tar Conc	Tar	ID(%) Tar Conc
Total	57	(100) 617.2	62	(100) 627.3	64	(100) 403.9	59	(100) 126.6	65	(100) 4548.2	67	(100) 1296.1
Entero-bacteriaceae	56	(98.2) 606.1	60	(96.8) 607.2	64	(100) 403.9	59	(100) 126.6	63	(96.9) 4407.2	66	(98.5) 1276.7
E. coli	39	(68.4) 422.2	36	(58.1) 364.5	45	(70.3) 283.9	44	(74.6) 94.4	34	(52.3) 2378.7	43	(64.2) 832.1
Klebsiella spp.	13	(22.8) 140.7	13	(21.0) 131.7	9	(14.1) 56.9	4	(6.8) 8.6	19	(29.2) 1328.1	14	(20.9) 270.9
Enterobacter spp.	4	(7.0) 43.2	8	(12.9) 80.9	6	(9.4) 38.0	5	(8.5) 10.8	7	(10.8) 491.2	6	(8.9) 115.4
Citrobacter spp.	-	3	(4.8)	30.1	2	(3.1) 12.5	5	(8.5) 10.8	3	(4.6) 209.2	1	(1.5) 19.4
Other	-	-	-	-	2	(3.1) 12.5	1	(1.7) 2.2	-	-	2	(3.0) 38.9
Citrobacter spp. or E. coli	-	-	-	-	-	-	-	-	-	-	-	-
Non-Entero-bacteriaceae	1	(1.8) 11.1	1	(1.6) 10.0	-	-	-	-	1	(1.5) 68.2	-	
G.L.L.S.	-	-	1	(1.6) 10.0	-	-	-	-	1	(1.5) 68.2	1	(1.5) 19.4

Tar = Number of colonies identified
Tar Conc = Concentration per 100 ml

TABLE 4:
Humber River/Black Creek Study: Dry Weather Results by Station m-TEC-IG

	H1		H2		H3		H4		B1		B2	
	Tar	ID(%)	Tar	ID(%)	Tar	ID(%)	Tar	ID(%)	Tar	ID(%)	Tar	ID(%)
Total	60	(100)	52	(100)	55	(100)	44	(100)	47	(100)	47	(100)
Entero-bacteriaceae	59	(98.3)	52	(100)	54	(98.2)	44	(100)	46	(97.9)	47	(100)
E. coli	53	(88.3)	47	(90.4)	50	(90.9)	37	(84.1)	39	(83.0)	41	(87.2)
Klebsiella spp.	1	(1.7)	2	(3.9)	-		1	(2.3)	6	(12.8)	2	(4.3)
Enterobacter spp.	1	(1.7)	2	(3.9)	3	(5.5)	-		1	(2.1)	-	
Citrobacter spp.	4	(6.6)	-		1	(1.8)	5	(11.4)	-		1	(2.1)
Other			1	(1.9			1	(2.3)			3	(6.4)
Citrobacter spp. or E. coli												
Non-Enterobacteriaceae	1	(1.7)			1	(1.8)						
G.L.L.S.									1	(2.1)		

Faecal Coliforms (m-TEC) Wet Weather Day 1 (Table 5)

The Humber River stations (H2 and H3) had a slightly higher %R of E. coli than the Black Creek stations (B1 and B2). However, in the Humber, the downstream station H2 had a lower recovery than H3 while in Black Creek the upstream station B2 had the lower recoveries. Both B1 and H2 appeared similar in the %R's of the different bacterial genera, while B2 which had the lowest %R of E. coli, had the highest recovery of Klebsiella spp., Enterobacter spp. and non-enterobacteriaceae. The concentrations of E. coli at the study sites decreased in the following order: B1>B2>H2>H3.

Faecal Coliforms (m-TEC) Wet Weather Day 2 (Table 6)

The results for the second wet weather event demonstrated a different pattern than the first. The %R of E. coli was highest at B1 where it was higher than during dry weather and lowest at H2. The H2 station was the only one that did not show an increase in percent E. coli from the previous survey. The highest recovery of Klebsiella spp. and Enterobacter spp. was again at the B2 station with high %R's also occurring at H2.

The relative concentrations of E. coli were lower than in the previous survey and this time H2 had a somewhat higher E. coli concentration than B2 The order of E. coli recovery from highest to lowest was: B1> H2>B2>H3.

Escherichia coli (M-TEC-IG) Wet Weather Day 1 (Table 7)

The %R of E. coli was higher on m-TEC-IG than on m-TEC and the %R decreased from upstream to downstream stations in the Humber while increasing in Black Creek. The actual E. coli concentrations were in good agreement with m-TEC, except at B2 where a higher %R resulted in the indicated concentration being almost double that shown on m-TEC The order of recovery was as follows: B1>B2> H2>H3.

TABLE 5:

Humber River/Black Creek Study: Wet Weather Results by Station
October Event (Day 1) m-TEC Agar

	H2		H3		B1		B2	
	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.
Total	43 (100)	1.28×10^4	39 (100)	4.15×10^3	40 (100)	5.53×10^4	38 (100)	2.63×10^4
Entero-bacteriaceae	39 (90.7)	1.16×10^4	38 (97.4)	4.04×10^3	40 (100)	5.53×10^4	36 (94.7)	2.49×10^4
E. coli	21 (48.8)	6.25×10^3	26 (66.7)	2.77×10^3	18 (45.0)	2.49×10^4	11 (28.9)	7.60×10^3
Klebsiella spp.	11 (25.6)	3.28×10^3	5 (12.8)	5.30×10^2	10 (25.0)	1.38×10^4	4 (10.5)	2.76×10^3
Enterobacter spp.	4 (9.3)	1.19×10^3	2 (5.1)	2.12×10^2	7 (17.5)	9.68×10^3	9 (23.7)	6.23×10^3
Citrobacter spp.	3 (7.0)	8.96×10^2	4 (10.2)	4.27×10^2	5 (12.5)	6.91×10^3	12 (31.6)	8.31×10^3
Other			1 (2.6)	1.08×10^2				
Non-Entero-bacteriaceae	- -	-	-	-	-	-	-	-
G.L.L.S.	4 (9.3)	1.19×10^3	1 (2.6)	1.08×10^2	-	-	2 (5.3)	1.39×10^3

TABLE 6:

Humber River/Black Creek Study: Wet Weather Results by Station
November Event (Day 2) m-TEC Agar

	H2		H3		B1		B2	
	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.
Total	40 (100)	8.75×10^3	47 (100)	3.57×10^3	62 (100)	1.56×10^4	66 (100)	5.19×10^3
Entero-bacteriaceae	39 (97.5)	8.53×10^3	43 (91.5)	3.27×10^3	60 (96.8)	1.51×10^4	66 (100)	5.19×10^3
E. coli	19 (47.5)	4.16×10^3	32 (68.1)	2.43×10^3	48 (77.4)	1.24×10^4	36 (54.6)	2.83×10^3
Klebsiella spp.	5 (12.5)	1.09×10^3	6 (12.8)	4.57×10^2	9 (14.5)	9.20×10^2	23 (34.9)	1.81×10^3
Enterobacter spp.	14 (35)	3.06×10^3	4 (8.5)	3.03×10^2	3 (4.8)	1.37×10^3	3 (4.5)	2.36×10^2
Citrobacter spp.	-	-	1 (2.1)	7.50×10^1	-		3 (4.5)	2.36×10^2
Other	-	-	-		-		-	
Citrobacter spp. of E. coli	1 (2.5)	2.18×10^2	-	-	-	-	1 (1.5)	7.79×10^1
Non-Entero-bacteriaceae	-	-	3 (6.4)	2.28×10^2	1 (1.6)	2.50×10^2	-	-
G.L.L.S.	1 (2.5)	2.18×10^2	1 (2.1)	7.50×10^1	1 (1.6)	2.50×10^2	-	-

TABLE 7:

Humber River/Black Creek Study: Wet Weather Results by Station
October Event (Day 1) m-TEC-IG Agar

	H2		H3		B1		B2	
	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.
Total	32 (100)	9.5×10^3	32 (100)	3.1×10^3	34 (100)	3.6×10^4	41 (100)	1.8×10^4
Entero-bacteriaceae	30 (93.8)	8.9×10^3	32 (100)	3.1×10^3	33 (97.1)	3.5×10^4	41 (100)	1.8×10^4
E. coli	26 (81.3)	7.7×10^3	28 (87.5)	2.7×10^3	27 (79.4)	2.9×10^4	31 (75.6)	1.4×10^4
Klebsiella spp.	1 (3.1)	2.9×10^2	-	-	2 (5.9)	2.1×10^3	1 (2.5)	4.3×10^2
Enterobacter spp.	2 (6.3)	5.9×10^2	1 (3.1)	9.60×10^1	3 (8.8)	3.2×10^3	1 (2.5)	4.3×10^2
Citrobacter spp.	1 (3.1)	2.9×10^2	3 (9.4)	2.9×10^2	1 (3.0)	1.0×10^3	6 (14.6)	2.6×10^3
Other	-	-	-	-	-	-	2 (4.9)	8.8×10^2
Citrobacter spp. or E. coli	-	-	-	-	-	-	-	-
Non-Entero-bacteriaceae	2 (6.3)	5.9×10^2	-	-	-	-	-	-
G.L.L.S.	-	-	-	-	1 (3.0)	1.0×10^3		

Escherichia coli (m-TEC-IG) Wet Weather Day 2 (Table 8)

The %R of E. coli was highest at H3 and B1 as during the first event and lowest at H2. The E. coli %R at H2 showed no increase from the first wet weather survey as on m-TEC; however, an increase in Klebsiella spp., Enterobacter spp. and non-enterobacteriaceae was apparent. During the second event, there was good agreement in levels of E. coli recovered from m-TEC and m-TEC-IG at all stations. Furthermore, a similar decrease in concentration from station to station was exhibited by both media: B1>H2>B2>H3.

Faecal Streptococci (m-Ent) Dry Weather (Table 9)

The %R of enterococci during dry weather conditions was highest at H3 and H2 and both stations also had the lowest non-faecal streptococcal recovery. The lowest %R's of enterococci were at station B2, primarily due to an increased recovery of S. avium. S. faecium variants comprised the majority of enterococci at all stations, although the %R's were somewhat lower in Black Creek. There was also a tendency for a shift from S. faecium var. casseliflavus to S. faecium between upstream (H3, H4 and B2) and downstream stations (H1, H2, and B1). The lowest %R of S. faecalis and its variants occurred at H2 mainly because of a decrease in the recovery of S. faecalis var. liquefaciens.

The relative levels of enterococci recovered at the six stations during dry weather surveys in decreasing order were: B1>B2>H2>H3>H4>H1.

TABLE 8:

Humber River/Black Creek Study: Wet Weather Results by Station
November Event (Day 2) m-TEC-IG Agar

	H2		H3		B1		B2	
	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.
Total	36 (100)	5.5×10^3	40 (100)	2.6×10^3	52 (100)	1.3×10^4	52 (100)	3.6×10^3
Entero-bacteriaceae	36 (100)	5.5×10^3	40 (100)	2.6×10^3	51 (98.1)	1.28×10^4	51 (98.1)	3.5×10^3
E coli	25 (69.4)	3.8×10^3	38 (95.0)	2.5×10^3	49 (94.2)	1.22×10^4	45 (86.6)	3.1×10^3
Klebsiella spp.	6 (16.7)	9.2×10^2	1 (2.5)	6.5×10^1	2 (3.9)	5.07×10^2	3 (5.8)	2.1×10^2
Enterobacter spp.	5 (13.9)	7.6×10^2	-	-	-	-	1 (1.9)	6.8×10^1
Citrobacter spp.	-	-	1 (2.5)	6.5×10^1	-	-	1 (1.9)	6.8×10^1
Other	-	-	-	-	-	-	1 (1.9)	6.8×10^1
Citrobacter spp. or E. Coli	-	-	-	-	-	-	-	
Non-Entero-bacteriaceae	-	-	-	-	-	-	-	
G.L.L.S.	-	-	-	-	1 (1.9)	2.5×10^2	1 (1.9)	6.8×10^1

ber River/Black Creek Study: Dry Weather Results by Station, m-Enterococcus Agar

H2		H3		H4		B1		B2	
ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.
5 (100)	$1.72X10^2$	67 (100)	$1.30X10^2$	71 (100)	$8.85X10^1$	80 (100)	$5.38X10^2$	70 (100)	$5.30X10^2$
2 (96.0)	$1.65X10^2$	64 (95.5)	$1.24X10^2$	62 (87.3)	$7.73X10^1$	72 (90.0)	$4.84X10^2$	60 (85.7)	$4.54X10^2$
1 (94.7)	$1.63X10^2$	64 (95.5)	$1.24X10^2$	60 (84.5)	$7.48X10^1$	69 (86.3)	$4.64X10^2$	52 (74.3)	$3.94X10^2$
-	-	1 (1.5)	1.95	-	-	2 (2.5)	$1.34X10^1$	-	-
2 (2.7)	4.59	3 (4.5)	5.85	2 (2.8)	2.49	5 (6.3)	$3.36X10^1$	4 (5.7)	$3.02X10^1$
3 (4.0)	6.88	6 (8.9)	$1.17X10^1$	7 (9.9)	8.73	7 (8.8)	$4.71X10^1$	6 (8.6)	$4.56X10^1$
1 (1.3)	2.29	2 (2.9)	3.77	-	-	1 (1.3)	6.73	1 (1.4)	7.42
9 (38.7)	$6.66X10^1$	18 (26.9)	$3.50X10^1$	16 (22.5)	$1.99X10^1$	32 (40.0)	$2.15X10^2$	15 (21.4)	$1.13X10^2$
3 (30.7)	$5.28X10^1$	28 (41.8)	$5.43X10^1$	26 (36.6)	$3.24X10^1$	6 (7.5)	$4.04X10^1$	15 (21.4)	$1.13X10^2$
3 (17.3)	$2.98X10^1$	6 (9.0)	$1.17X10^1$	9 (12.7)	$1.12X10^1$	16 (20)	$1.06X10^2$	11 (15.7)	$8.32X10^1$
1 (1.3)	2.29	-	-	2 (2.8)	2.49	3 (3.8)	$2.02X10^1$	8 (11.4)	$6.04X10^1$
-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-
1 (1.3)	2.29	-	-	2 (2.8)	2.49	3 (3.8)	$2.02X10^1$	8 (11.4)	$6.04X10^1$
-	-	-	-	-	-	-	-	-	-
3 (4.0)	6.88	3 (4.5)	5.85	9 (12.7)	$1.12X10^1$	8 (10.0)	$5.38X10^1$	10 (14.3)	$7.58X10^1$

Enterococci (m-ME) Dry Weather (Table 10)

The %R of enterococci was less variable on m-ME than on m-Ent agar during dry weather, and where lower %R's where noted it was due to the presence of S. bovis which was not isolated on m-Ent agar.

There was again a tendency toward higher %R's of S. faecium var. casseliflavus at the upstream stations, but overall the recoveries were much lower than on m-Ent. The %R's of S. faecium were somewhat higher on m-ME at all stations except H4 which had the highest recovery of S. faecalis var. liquefaciens (42.9%) for either streptococcus media during wet and dry weather surveys.

The concentrations of enterococci as determined by the m-ME procedure were significantly lower than those indicated by the identification of target colonies from m-Ent agar. The relative concentrations in decreasing order were similar to m-Ent results (i.e. B1 > B2 > H1 > H2 > H3 > H4), with the exception that higher recoveries were obtained at station H1.

Faecal Streptococci (m-Ent) Wet Weather Day 1 (Table 11)

During wet weather events, the %R of enterococci from m-Ent increased in the downstream stations on both the Humber River and Black Creek, while the %R's of non-faecal streptococci decreased. There was also a shift in the streptococcal populations at the lower stations with S. faecalis increasing overall due to higher recoveries of S. faecalis var. liquefaciens and S. faecium decreasing overall with a change in %R's from S. faecium var. casseliflavus to S. faecium. In addition, S. durans increased in a downstream direction.

The concentration of enterococci was considerably increased over dry weather levels, particularly in Black Creek. The relative concentrations in decreasing order were: B2 > B1 > H2 > H3.

Faecal Streptococci (m-Ent) Wet Weather Day 2 (Table 12)

The %R of enterococci from Black Creek was again higher at the downstream location (B1); however, the population distribution was different from the first wet

umber River/Black Creek Study: Dry Weather Results by Station, m-ME Agar

H2		H3			H4			B1			B2	
ID(%)	Tar Conc.	Tar	ID(%)	Tar Conc.	Tar	ID(%)	Tar Conc.	Tar	ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.
100)	$1.17X10^{1}$	55	(100)	9.0	21	(100)	2.3	68	(100)	$8.79X10^{1}$	61 (100)	$3.55X10^{1}$
96.8)	$1.13X10^{1}$	54	(98.2)	8.84	20	(95.2)	2.19	66	(97.1)	$8.54X10^{1}$	58 (95.1)	$3.38X10^{1}$
95.2)	$1.11X10^{1}$	50	(90.9)	8.18	18	(85.7)	1.97	66	(97.1)	$8.54X10^{1}$	57 (93.4)	$3.32X10^{1}$
1.6)	$1.87X10^{-1}$	1	(1.8)	0.16	-		-	1	(1.5)	1.32	-	-
6.3)	$7.49X10^{-1}$	4	(7.3)	0.65	2	(9.5)	0.22	2	(2.9)	2.55	2 (3.3)	1.17
(9.5)	1.11	6	(10.9)	0.98	9	(42.9)	0.98	7	(10.3)	9.05	7 (11.5)	4.08
(1.6)	$1.87X10^{-1}$	-		-	-		-	-		-	-	-
(52.4)	6.13	22	(40.0)	3.6	2	(9.5)	0.22	35	(51.5)	$4.53X10^{1}$	29 (47.5)	$1.69X10^{1}$
(14.3)	1.67	8	(14.5)	1.31	4	(19.0)	0.44	7	(10.3)	9.05	9 (14.8)	5.25
(9.5)	1.11	9	(16.4)	1.47	1	(4.8)	0.11	14	(20.6)	$1.81X10^{1}$	10 (16.4)	5.82
(1.6)	$1.87X10^{-1}$	4	(7.3)	0.65	2	(9.5)	0.22	-		-	1 (1.6)	0.582
	-	3	(5.5)	0.49	2	(9.5)	0.22	-		-	1 (1.6)	0.582
	-	1	(1.8)	0.16	-		-	-		-	-	-
(1.6)	$1.87X10^{-1}$	-		-	-		-	-		-	-	-
	-	-		-	-		-	-		-	-	-
(3.2)	$3.74X10^{-1}$	1	(1.8)	0.16	1	(4.8)	0.11	2	(2.9)	2.55	3 (4.9)	1.74

TABLE 11: Humber River/Black Creek Study: Wet Weather Results by Station
October Event (Day 1) m-Enterococcus Agar

	H2		H3		B1		B2	
	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.
Total	50 (100)	4.84×10^3	46 (100)	2.85×10^3	59 (100)	2.27×10^4	63 (100)	5.71×10^4
Faecal Streptococcus	44 (88)	4.26×10^3	35 (76.1)	2.17×10^3	47 (79.7)	1.81×10^4	48 (76.2)	4.35×10^4
Enterococci	41 (82)	3.97×10^3	33 (71.7)	2.04×10^3	46 (78.0)	1.77×10^4	47 (74.6)	4.26×10^4
S. faecalis	1 (2.0)	9.7×10^1	3 (6.5)	1.86×10^2	0 (0)	0	1 (1.6)	9.06×10^2
Var. faecalis	6 (12.0)	5.81×10^2	4 (8.7)	2.48×10^2	9 (15.3)	3.46×10^3	10 (15.9)	9.06×10^3
Var. liquefaciens	17 (34)	1.65×10^3	5 (10.9)	3.09×10^2	12 (20.3)	4.62×10^3	8 (12.7)	7.25×10^3
Var. zymogenes	1 (2.0)	9.7×10^1	0 (0)	0	0 (0)	0	0 (0)	0
S. faecium	11 (22.0)	1.06×10^3	6 (13.0)	3.72×10^2	14 (23.7)	5.39×10^3	9 (14.3)	8.16×10^3
Var. casseliflavus	0 (0)	0	12 (26.1)	7.43×10^2	5 (8.5)	1.92×10^3	14 (22.2)	1.27×10^4
S. durans	5 (10.0)	4.84×10^2	3 (6.5)	1.86×10^2	6 (10.2)	2.31×10^3	5 (7.9)	4.53×10^3
Non- Enterococci	3 (6.0)	2.90×10^2	2 (4.3)	1.24×10^2	1 (1.7)	3.85×10^2	1 (1.6)	9.06×10^2
S. bovis	0 (0)	0	1 (2.2)	6.19×10^1	0 (0)	0	0 (0)	0
S. bovis (Var.)	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0
S. avium	3 (6.0)	2.90×10^2	1 (2.2)	6.19×10^1	1 (1.7)	3.85×10^2	1 (1.6)	9.06×10^2
S. equinus	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)	0
Non-faecal streptococci	6 (12.0)	5.81×10^2	11 (23.9)	6.81×10^2	12 (20.3)	4.62×10^3	15 (23.8)	1.36×10^4

TABLE 12: Humber River/Black Creek Study: Wet Weather Results by Station
November Event (Day 2) m-Enterococcus Agar

	H2		H3		B1		B2	
	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.
Total	55 (100)	4.71×10^3	44 (100)	2.31×10^3	78 (100)	9.97×10^3	76 (100)	4.93×10^3
Faecal streptococci	48 (87.3)	4.11×10^3	40 (90.9)	2.10×10^3	71 (91.0)	9.07×10^3	67 (88.2)	4.35×10^3
Enterococci	45 (81.8)	3.85×10^3	37 (84.1)	1.94×10^3	67 (85.9)	8.56×10^3	58 (76.3)	3.76×10^3
S. faecalis	1 (1.8)	8.56×10^1			1 (1.3)	1.28×10^2		
Var. faecalis			1 (2.3)	5.25×10^1	13 (16.7)	1.66×10^3	3 (3.9)	1.95×10^2
Var. liquefaciens	10 (18.2)	8.56×10^2	9 (20.4)	4.72×10^2	7 (9.0)	8.95×10^2	8 (10.6)	5.19×10^2
Var. zymogenes								
S. faecium	15 (27.3)	1.28×10^3	7 (15.9)	3.67×10^2	14 (17.9)	1.79×10^3	18 (23.7)	1.17×10^3
Var. casseliflavus	10 (18.3)	8.56×10^2	9 (20.4)	4.72×10^2	11 (14.1)	1.41×10^3	12 (15.8)	7.78×10^2
S. durans	9 (16.4)	7.71×10^2	11 (25.0)	5.77×10^2	21 (26.9)	2.68×10^3	17 (22.4)	1.10×10^3
Non-Enterococci	3 (5.5)	2.57×10^2	3 (6.8)	1.57×10^2	4 (5.1)	5.11×10^2	9 (11.8)	5.84×10^2
S. bovis	1 (1.8)	8.56×10^1	1 (2.3)	5.25×10^1	1 (1.3)	1.28×10^2	2 (2.6)	1.30×10^2
S. bovis (Var.)								
S. avium	2 (3.6)	1.71×10^2	2 (4.6)	1.05×10^2	3 (3.8)	3.73×10^1	7 (9.2)	4.54×10^2
S. equinus								
Non-faecal streptococci	7 (12.7)	5.99×10^2	4 (9.1)	2.10×10^2	7 (9.0)	8.95×10^2	9 (11.8)	5.84×10^2

weather survey. The %R of S. faecalis variants had dropped slightly, while S. durans and non-enterococci had increased. In the Humber River the %R of enterococci decreased slightly at the downstream station H2 and both S. faecalis and S. durans were recovered at lower percentages in comparison with H3.

The relative concentrations of enterococci were changed from the first wet weather results. The levels in decreasing order for the second event were: B1 > H2>B2>H3.

Enterococci (m-ME) Wet Weather Day 1 (Table 13)

The %R of enterococci on m-ME decreased at the downstream stations (H2 and B1), constituting a reverse of the results obtained on m-Ent and, in addition, there was a shift toward higher %R's of S. faecium variants on m-Me. S. avium was not recovered on m-ME except at station B1, while it was recovered at all stations on m-Ent. S. bovis in turn comprised a higher percentage of the target colonies on m-ME than on m-Ent.

The %R's of S. faecium var. casseliflavus decreased at the downstream stations as was the case with m-Ent, however, S. faecium also showed a decrease which was not evident on m-Ent.

S. faecalis var. liquefaciens increased form B2 to B1 but showed a decrease at the Humber River downstream station H2. The %R of S. durans was also higher in Black Creek than in the Humber River, but increased in the downstream stations in both cases.

Overall, the concentration of enterococci in the Humber River and Black Creek demonstrated a considerable increase over the dry weather observations and the ratio of actual enterococcus levels determined by m-ME increased relative to that indicated my m-Ent. The relative concentrations determined on m-ME in decreasing order were: B2>B1>H2>H3.

TABLE 13: Humber River/Black Creek Study: Wet Weather Results by Station
October Event (Day 1) m-ME Agar

	H2		H3		B1		B2	
	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.
Total	42 (100)	3.51×10^3	38 (100)	9.29×10^2	40 (100)	7.98×10^3	39 (100)	7.36×10^3
Faecal streptococci	38 (90.5)	3.18×10^3	37 (97.4)	9.04×10^2	37 (92.95)	7.38×10^3	35 (89.7)	6.60×10^3
Enterococci	35 (83.3)	2.92×10^3	35 (92.1)	8.56×10^2	31 (77.5)	6.18×10^3	33 (84.6)	6.23×10^3
S. faecalis	-	-	-	-	-	-	1 (2.6)	1.89×10^2
Var. faecalis	6 (14.3)	5.01×10^2	6 (15.8)	1.47×10^2	2 (5.0)	3.99×10^2	1 (2.6)	1.89×10^2
Var. liquefaciens	5 (11.9)	4.18×10^2	6 (15.8)	1.47×10^2	5 (12.5)	9.97×10^2	3 (7.7)	5.66×10^2
Var. zymogenes	1 (2.4)	8.36×10^1	-	-	-	-	1 (2.6)	1.89×10^2
S. faecium	12 (28.6)	1.00×10^3	14 (36.8)	3.42×10^2	11 (12.5)	2.19×10^3	15 (38.5)	2.83×10^3
Var. casseliflavus	7 (16.7)	5.85×10^2	9 (23.7)	2.20×10^2	6 (15.0)	1.20×10^3	8 (20.5)	1.51×10^3
Non-enterococci	3 (7.1)	2.51×10^2	2 (5.3)	4.89×10^1	6 (15.)	1.20×10^3	2 (5.1)	3.77×10^2
S. durans	4 (9.5)	3.34×10^2	-	-	7 (17.5)	1.40×10^3	4 (10.2)	7.55×10^2
S. bovis	3 (7.1)	2.51×10^2	1 (2.6)	2.44×10^1	4 (100)	7.98×10^2	2 (5.1)	3.77×10^2
S. bovis (Var.)			1 (2.6)	2.44×10^1	-	-	-	-
S. avium	-	-	-	-	2 (5.0)	3.99×10^2	-	-
S. equinus	-	-	-	-	-	-	-	-
Non-faecal streptococci	4 (9.5)	3.34×10^2	1 (2.6)	2.44×10^1	3 (7.5)	5.98×10^2	4 (10.2)	7.55×10^2

Enterococci (m-ME) Wet Weather Day 2 (Table 14)

The %R of enterococci on m-ME was slightly higher during the second wet weather survey, with a similar tendency for a decrease at the downstream stations observed during the first event.

The %R of S. faecalis variants was somewhat lower during this wet event compared with the previous one and showed little change between upstream and downstream stations on both the Humber River and Black Creek. There was also a change in the variant compositon of the S. faecalis population. The %R's of S. faecium variants were about the same at B2 and H3, but rose at B1 and H2 in comparison to the first event. This resulted in small concentration increases in the downstream stations as opposed to a larger increase in the Humber and a decrease in Black Creek that were noted during the first wet weather survey. S. faecium again decreased in relative proportion at the Humber River downstream station but showed no change downstream in Black Creek while S. faecium var. casseliflavus increased in both bodies of water. The %R's of S. durans decreased downstream (H2 and B1). Somewhat lower %R's of faecal streptococci (non enterococccci) were noted at stations H2, H3 and B1 than during the first wet weather survey.

The geometric mean concentrations of enterococci, determined using m-ME media, were slightly lower during this event at station H2, but showed an increase at the other stations.

A major difference between this survey and the previous wet and dry weather surveys was that the actual concentration of enterococci, as determined by the identification of bacteria isolated form m-ME, was higher than that from m-Ent and in relatively good agreement. The relative concentrations determined on m-ME in decreasing order were: B2>B1>H2>H3.

TABLE 14: Humber River/Black Creek Study: Wet Weather Results by Station
November Event (Day 2) m-ME Agar

	H2		H3		B1		B2	
	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.	Tar ID(%)	Tar Conc.
Total	35 (100)	4.41×10^3	39 (100)	2.69×10^3	49 (100)	1.26×10^4	54 (100)	4.63×10^3
Faecal streptococci	32 (91.4)	4.28×10^3	38 (97.4)	2.62×10^3	47 (95.9)	1.21×10^4	54 (100)	4.63×10^3
Enterococci	32 (91.4)	4.03×10^3	38 (97.4)	2.62×10^3	45 (91.8)	1.16×10^4	51 (94.4)	4.37×10^3
S. faecalis	1 (2.8)	1.26×10^2	-	-	-	-	-	-
Var. faecalis	-	-	2 (5.2)	1.38×10^2	2 (4.1)	5.14×10^2	2 (3.7)	1.71×10^2
Var. liquefaciens	7 (20.0)	8.82×10^2	7 (17.9)	4.83×10^2	5 (10.2)	1.29×10^3	5 (9.3)	4.29×10^2
Var. zymogenes	-	-	-	-	-	-	1 (1.9)	8.57×10^1
S. faecium	9 (25.7)	1.13×10^3	15 (38.5)	1.03×10^3	20 (40.8)	5.14×10^3	22 (40.7)	1.89×10^3
Var. casseliflavus	12 (34.3)	1.51×10^3	7 (17.9)	4.83×10^2	11 (22.5)	2.83×10^3	11 (20.4)	9.43×10^2
S. durans	3 (8.6)	3.78×10^2	7 (17.9)	4.83×10^2	7 (14.3)	$1.80\ 10^3$	10 (18.5)	8.57×10^2
F. Streptococci Non-enterococci	2 (5.7)	2.52×10^2	-	-	2 (4.1)	5.14×10^2	3 (5.6)	2.57×10^2
S. bovis	2 (5.7)	2.52×10^2	-	-	1 (2.0)	2.57×10^2	3 (5.6)	2.57×10^2
S. bovis (Var.)	-	-	-	-	-	-	-	-
S. avium	-	-	-	-	1 (2.0)	2.57×10^2	-	-
S. equinus	-	-	-	-	-	-	-	-
Non-faecal streptococci	1 (2.9)	1.26×10^2	1 (2.6)	6.90×10^1	2 (4.1)	5.14×10^2	-	-

Discussion

Specificity and Selectivity of m-TEC-IG and m-ME

The specificty and selectivity indices of m-TEC-IG and m-ME are shown in Table 15. The data used for determining the performance indices for m-TEC-IG and m-ME are documented in Table 1 (m-TEC-IG) and Table 2 (m-ME). Data for m-TEC and m-Enterococcus agar are also presented in the above two tables for comparative purposes.

In both cases it can be seen that a higher percentage of the appropriate target organisms are isolated on the newer media. There are also some differences noted in the relative proportion of different genera and species isolated. In the case of m-TEC-IG this is primarily due to its increased specificity for E. coli because of the addition of indoxyl-β-D-glucoside (IG) to the medium. The increased specificity of m-ME for enterococci is also due to the addition of IG plus a completely different medium formulation (see Appendix A) and a higher (41.5°C) incubation temperature.

Specificity is a measure of how well a medium isolates and identifies the presence of target organisms and inhibits non-target organisms. Specificity is made up of 2 components:

1) False positive error, which is calculated by dividing the number of false positive target colonies by the total number of presumptive target colonies.
2) Undetected target error, which is determined by dividing the number of undetected target (false negative) colonies by the sum of the number of verified target colonies and the undetected target colonies.

The selectivity index is calculated by dividing the total number of presumptive target colonies by the total number of both presumptive target and presumptive non-target colonies.

TABLE 15: Verification of Presumptive Target and Non-Target Colonies for m-TEC-IG and m-ME - Specificity and Selectivity Indices

Performance Parameter	Number of Colonies on			
	m-TEC-IG		m-ME	
	Dry	Wet	Dry	Wet
Presumptive target	305	319	321	337
Verified target	267	269	302	301
Presumptive non-target	161	188	71	97
Verified non-target	141	162	23	40
Specificity Indicies (%)				
1. False Positive Error	$\frac{38}{305} \times 100 = 10.8$	$\frac{50}{319} \times 100 = 15.7$	$\frac{19}{321} \times 100 = 5.9$	$\frac{36}{337} \times 100 = 10.7$
2. Undectected target error	$\frac{20}{287} \times 100 = 7.0$	$\frac{26}{295} \times 100 = 8.8$	$\frac{48}{350} \times 100 = 13.7$	$\frac{57}{358} \times 100 = 15.9$
Selectivity Index (%)	$\frac{305}{466} \times 100 = 65.6$	$\frac{319}{507} \times 100 = 62.9$	$\frac{321}{392} \times 100 = 81.9$	$\frac{337}{434} \times 100 = 77.6$

m-TEC-IG Performance Evalutation

The specificity indices under dry weather sampling conditions were acceptable. During wet weather surveys both specificity indices increased, particularly the false positive error which reached the limit of acceptability. It would appear that during wet weather events there were a higher number of non-E. coli faecal coliforms that were incapable of metabolizing indoxyl-β-D-glucoside and therefore did not produce the blue halo that distinguishes them as background colonies. The reason for this is unknown but may be due to the presence, during storm events, of a larger percentage of environmentally stressed organisms with damaged enzyme systems. This problem might be eliminated by retaining the urease test used with standard m-TEC agar (Dufour 1981) or incorporating a small concentration of urea into the agar.

The fact that there were numerous false negative or undetected E. coli colonies is puzzling. E. coli does not have the enzyme system required to metaboilize the indoxyl-β-D-glucoside indicator to produce a blue halo, however, it is possible that the false negative E. coli colonies could appear to have a halo if they were in close proximity to other faecal coliform colonies that produced strong colour (halo) reactions. The problem was compounded by the fact that 30% or more of the colonies on each filter were non-target as shown by the selectivity index.

The relatively low selectivity of m-TEC-IG is a consequence of it being a faecal coliform medium (m-TEC) with an indicator (indoxyl-β-D-glucoside) to differentiate E. coli from other faecal coliforms. Since the non-E. coli faecal coliforms can still grow on the medium, the number of non-target colonies is increased in comparison with the original faecal coliform medium (m-TEC) which had a selectivity index of 86%. The low selectivity index of m-TEC-IG may also have an effect on the accuracy of the counts as the number of colonies increase. The Environmental Protection Agency (E.P.A.) in the United Statles recommends an upper limit of 75 target colonies for the counting range of m-TEC-IG (Dufour 1985 personal communication). This is much lower than the 150 target colony limit for m-TEC used by the Ministry of the Environment and counting range experiments should be conducted on the new medium.

Despite the difficulties indicated, the m-TEC-IG medium provides a good estimate of the concentration of E. coli present in a sample. There is a tendency toward a slight over-estimation of the actual E. coli colonies present, i.e. 6.3% under dry weather conditions and 8.1% during wet weather. However, this would result in a negligible impact on water quality interpretation.

m-ME Performance Evaluation

The false positive error obtained is acceptable under both dry and wet conditions; however, the undetected target error was somewhat higher than expected. This may be due to the stringent counting procedures that were adopted because the medium was new and untested. Colonies, regardless of colour, were counted as non-target if no blue halo, signifying the metabolism of indoxyl-β-D-glucoside, was present. Some of the non-target colonies were blue which may have been due to a weak metabolism of IG (not strong enough to produce a halo around the colony). These blue non-target colonies may have in fact been enterococci with weak enzyme systems. Halos produced on m-ME varied in shade from light to dark blue indicating various levels of IG metabolism among the enterococcal species. Further investigation of non-haloed and faintly haloed colonies should be undertaken to determine if they should be included in the target count.

The selectivity index of m-ME is acceptable under both types of weather conditions. This indicates less potential interference from non-target colonies. The E.P.A. recommends a somewhat lower counting range for this medium than used in Ontario and counting range experiments should be conducted to establish its upper limit.

The m-ME medium appears to be acceptable for assessment of the concentration of enterococci in surface waters. The performance of the medium indicates a small underestimation of the actual number of enterococcal colonies present on a filter, i.e. 8.3% under dry weather conditions and 5.9% during storm events. This would not have a significant effect on the interpretation of surface water quality, but it is perhaps better to overestimate the concentration of the indicator organism than to underestimate it and thus err on the side of safety.

With the possible exception of some industrial wastes, e.g. pulp and paper mills, the Humber River provides as severe a test of a medium as any surface water body. The quantity and number of different inputs, both faecal and non-faecal, contributing to the river, particularily during storm events, represents a very complex survey situation. Under survey conditions in which a body of water is impacted by a more limited number of sources, the performance of both m-TEC-IG and m-ME could be expected to improve.

Levels of Faecal Pollution in the Humber River/Black Creek

The bacterium Esherichia coli is usually the predominant faecal coliform found in the faeces of both humans and animals. Unlike other members of the faecal coliform group, it cannot be isolated from environmental sources. Once E. coli from faecal material has been deposited in an environment such as surface waters it is incapable of growing or even surviving for long periods of time (there is approximately a 90% die-off rate within 24 hours in a nutrient-deficient environment (Sjogren and Gibson 1980).

The relative contribution of faeces to the faecal coliform population in a body of water can thus be assessed by the percent E. coli present as target colonies on m-TEC medium (the m-TEC-IG results cannot be used in this way because the medium is biased towards the recovery of E. coli). An increasing correlation between the target colony densities on both media, especially when coupled with a decrease in the background colony counts, may indicate a shift towards increased faecal contamination since both m-TEC and m-TEC-IG exhibit closely related counts when used in faecal sample analysis. (P. Seyfried, E. Harris and M. Young, University of Toronto Faecal Study, in progress, 1985).

The percentage of enterococci present as target colonies on either m-Ent or m-ME can best be used to provide back-up information for the m-TEC data. Both media have a high specificity for enterococci isolated from the faeces of humans and animals. However, some enterococcal species may enter surface waters from non-faecal sources e.g. plants (Mundt 1962, Langston 1960) and may persist in the environment for longer periods of time than E. coli (McFeters et. al. 1973). These occurrences could result in the false enumeration of faecal streptococcal densities in surface waters or misinterpretation of the data obtained.

The enterococcus isolation procedure using m-ME is a harsher technique compared with the use of m-Ent since the medium has fewer available nutrients and is incubated at a higher temperature. This may be responsible for the lower recoveries of enterococci on m-ME during dry weather than on m-Ent and the more closely related recoveries during wet weather. Enterococci that are not from recent faecal inputs could be stressed by their contact with the surface water

environment and as a result would be less able to grow on m-ME agar. Thus an increasing ratio of enterococci recovered on m-ME with respect to the recoveries on m-Ent may signify an increasing level of recent faecal contamination (i.e during a storm). The ability of m-ME to recover certain species of faecal streptococci that are less hardy (i.e. S. bovis) better than M-Ent agar adds to this theory since they would have had to enter the surface water fairly soon before sampling.

Interpretation of Dry Weather Survey Bacterial Concentrations

On a station by station basis, the E. coli results demonstrate faecal pollution inputs to be considerably higher in Black Creek than in the Humber River, particularily at station B1 (Tables 3 and 4). In both bodies of water, the concentration of E. coli (m-TEC and m-TEC-IG results) increased in a downstream direction indicating continued "fresh" inputs on an increasing scale. The percent recovery of E. coli from both media demonstrates the tendency towards increased loadings of non-E. coli faecal coliforms at the downstream stations. This could be due to the high survival rate of these organisms from upstream inputs and/or from stirred-up sediments at the downstream locations (Niemela and Vaatamen 1982, Sjogren and Gibson 1981, Matson et. al. 1978).

The high percent recoveries of enterococci from both m-Ent and m-ME agar (Tables 9 and 10) and the tendency towards increasing concentrations at the downstream stations confirms the analysis of the E. coli data with the only exception being the faecal streptococcal and enterococcal levels recorded at station H1 from both media. It is possible that the major sources contributing to faecal pollution have changed to ones that have relatively lower levels of faecal streptococci as indicated by the drop in concentration of faecal streptococci from H2 to H1. The slight increase in the percent recovery of non-faecal streptococci at H1 could also be due to sediment stir up or direct input of non-faecal material which would affect a drop in the recovery of faecal streptococci.

Overall, during dry weather conditions a consistantly high concentration and percent recovery of E. coli and enterococci at any given station must be related to the discharge of human faecal material into the water from nearby point sources,

(i.e. storm sewers with illegal sanitary connnections) and/or the direct input of animal faecal wastes.

Interpretation of Wet Weather Survey Bacterial Concentrations

The data obtained during wet weather surveys, with few exceptions, show lower percent recoveries of E. coli (Tables 5, 6, 7 and 8) in the Humber River and Black Creek then during dry weather. This trend towards a higher proportional population of other faecal coliforms, particularily Enterobacter spp. and Citrobacter spp., is the result of increased inputs of non-faecal and/or distant faecal sources. One exception to the decrease in proportional recovery of E. coli occurred during the second event at station B1 (m-TEC and m-TEC-IG) where the percent recovery of E. coli actually increased. It is possible that this second event, which lasted longer than the first resulted in a larger impact on B1 from the combined sewers upstream, overriding the effect of "natural" storm drainage. A slightly higher percent recovery of E. coli was also noted at H3 on m-TEC-IG but not on m-TEC. Further investigation would be required to determine the cause. All stations except H2 showed a higher percent E. coli recovery during the second storm event, than during the first, possibly indicating proportionally greater inputs of fresh faecal material.

Part of the increased faecal input during the second event may come from further upstream; the increased flow rate in the river would allow E. coli from faecal material to travel further and the colder temperatures would slow down the rate of die-off. A shorter period of accumulation of faecal material flushed in and colder air temperatures may also effect the relative impact of fresh faeces by reducing the build-up of old faeces and slowing the die-off of bacteria in the faeces.

Station H2 and H3 have approximately the same percent recovery of E. coli (m-TEC) during both storm events indicating approximately the same proportional loadings of fresh faecal wastes and other inputs. The relative change in proportion of non-E. coli faecal coliforms during the second event at H2, i.e. a decrease in Klebsiella spp. and increase in Enterobacter spp. may indicate a larger input of "natural" (non-faecal) as opposed to distant faecal material and this could be related

to the longer period of rainfall and therefore runoff that occurred with the second wet weather survey. H3 which actually had an E. coli percent recovery 1.7% higher than during the first event, did not exhibit any significant changes in non-E. coli recoveries.

The geometric mean concentrations of E. coli did not increase as much during the second event as the first. This was more noticeable in Black Creek where counts were less than half those obtained during the first wet weather survey. This may again be the result of increased input from non-faecal sources or the prolonged rainfall causing greater dilution over the event.

There was also a tendency for higher concentrations to be reached on a sample to sample basis during the first event which would obviously contribute to a higher geometric mean. If there was less build-up of fresh faecal material prior to the second wet weather survey, this would result in lower bacterial concentrations. Total faecal loadings could, however, be higher over the duration of the second event.

The changes in proportional levels of enterococci and other faecal streptococci supports the faecal coliform/E. coli results. The tendency is towards lower enterococcal and higher non-faecal streptococcal percent recoveries during the wet events. The trends noted were, however, not as dramatic as with the faecal coliform data for the reasons stated earlier (i.e. relatively high specificity for enterococci and recovery of "non-faecal" enterococci.

During the first wet weather survey (Tables 11 and 13), the non-faecal streptococci increased their representation on both media which would also be indicative of an impact from non-faecal sources. (A drop in the enterococcus percent recovery on m-Ent did not occur at B2.)

During the second wet weather event (Tables 12 and 14), B1 and B2 (m-Ent) and H3 and B2 (m-ME) demonstrated similar or slightly higher enterococcal recovery than during dry weather as well as a small increase in non-faecal streptococci (except H3). These percent recoveries were higher than the first event as were those at H3 (m-Ent) and H2 (m-ME). The streptococcal population shifts would support the E. coli data that indicate an increased proportion of fresh faecal input

during the second wet weather survey.

The tendency for an increase in the proportion of enterococci that are Streptococcus faecalis varieties and a relative decrease in the Streptococcus faecium varieties may also indicate impacts from more recent faecal pollution because S. faecalis tends to die off faster in polluted wates than S. faecium (Dufour personal communication, 1985). This could be somewhat offset during the higher flows of storm events by faecal input upstream, although the nature of the original input and its distance upstream would still play a role.

The fact that there was higher, recent faecal pollution of the HumberRiver/Black Creek survey area during storm events was evidenced by the increased E. coli concentrations and was also confirmed by higher levels of enterococci as determined by both m-Ent and m-ME (Tables 11 - 14). The high concentration of enterococci isolated on m-ME at B1 may be a consequence of the combined sewer inputs.

Overall the wet weather surveys tended to yield decreases in the percent recoveries of E. coli and enterococci (Tables 1 and 2) and, as a result, the detection of faecal input could be obscurred. This is not an unexpected occurrence since, during wet weather events, there is an increased input from storm sewers and direct land run-off which results in inputs of non-faecal microbial populations to the river as well as increased flows causing impact on a given point from more distant sources.

Sources of Faecal Pollution in the Humber River/Black Creek during Dry Weather

Dry weather faecal inputs impacting on the survey area are both direct, in the form of non-human faeces and indirect, human inputs due to illegal connections to storm sewers. Added to these are some non-faecal inputs from run-off into storm sewer lines. There should be minimum input of non-faecal streptococci and faecal streptococci from vegetation.

Identification of streptococcal isolates demonstrated a predominance of S. faecium varieties in both Black Creek and the Humber River. The one exception was at the H4 site using m-ME agar (Table 10).

Examination of the species breakdown on the two streptococci media showed that not only were recovery levels different, but the proportions of the different bacterial isolates were also different. The m-ME medium gave higher percent recoveries of S. faecium varieties in Black Creek, while m-Ent agar showed this to be the case in the Humber River. The possibility discussed earlier that m-ME reflects a more recent faecal pollution input could be responsible for the variations in the proportions of streptococcal species recovered by the two media.

The higher levels of S. faecium could, in part, be due to its better survival in the river, but inputs of human faeces can have a high proportion of S. faecium (Table 16). Gulls, dogs, ducks and geese could also be contributing. The m-ME and m-Ent agar both showed an increased percent recovery of S. faecium in a downstream direction (B2 B1 and H4 H2) with station H1 showing a relative decrease in S. faecium and an increase in S. faecalis var. liquefaciens. This may indicate increased downstream human faecal loadings with very recent faecal inputs at H1 from both human and non-human sources. However, the occurrence of higher levels of non-faecal streptococci at H1 on m-Ent agar (Table 9) indicates environmental inputs as well. S. faecalis var liquefaciens may also be isolated from plant material (Mundt, Coggin and Johnson 1962), but this should not effect it is recovery during dry weather conditions. Representative populations of Streptococcus faecium on m-ME are indicative of potentially higher inputs of human faeces per unit volume to Black Creek.

S. faecium var. casseliflavus is indicative of non-human pollution and is recovered primarily from geese with small recoveries from muskrats and ducks. S. faecium var. casseliflavus can also come from "natural" sources (Mundt and Graham 1968), but this should not cause increased recoveries during dry weather. The decrease in a downstream direction may signify a decreasing proportion of non-human faecal inputs. The slight jump in recovery at station H3 on m-Ent agar may be due to the goose population at James Gardens. Further bacteriological investigations of this area are underway as part of a RAC project at the University of Toronto, under the direction of P. Seyfried.

The presence of S. bovis (m-ME) at upstream locations on both the Humber River and Black Creek could be indicative of agricultural inputs (i.e. cattle) further upstream (Seeley and Dain 1959). However, since S. bovis dies off rapidly in surface waters (Geldreich 1976), its presence is more likely an indication of pollution from dogs or possibly gulls (Table 16) at the upstream sites (H3, H4, B2).

TABLE 16: Percent Species of Fecal Streptococci In Human and Non-Human Feces from Target Colonies on m-Enterococcus m-ME and KF Agar Isolated Before Stress (1984)

SOURCE	TOTAL ISOLATES	S. FAECALIS VAR. LIQ	FAECALIS	ZYM	S. FAECIUM VAR. FAECIUM	CASSELIFLAVUS	S. DURANS	S. BOVIS	S. BOVIS VAR.	S. EQUINUS	S.AVIUM	AEROCOCCUS	F.S.*	NON + F.S.	STAPHYLOCOCCI
Humans	188	38 (20)	6 (3.2)	2 (1.1)	79 (42)	-	42 (22.3)	5 (2.65)	1 (0.5)	-	-	-	3 (1.6)	9 (4.8)	3 (1.6)
Gulls	227	89 (39.2)	9 (4.0)	17 (7.5)	38 (16.7)	16 (7.0)	8 (3.5)	33 (14.5)	1 (0.4)	-	1 (0.4)	-	-	10 (4.4)	5 (2.2)
Ducks	194	56 (28.9)	19 (9.8)	8 (4.1)	57 (29.4)	19 (9.8)	18 (9.3)	-	-	-	-	1 (0.5)	2 (1.0)	2 (1.0)	12 (6.2)
Geese	255	63 (24.7)	8 (3.1)	2 (0.8)	35 (13.7)	100 (39.2)	43 (16.9)	-	-	-	-	-	-	-	4 (1.6)
Pigeons (Domestic)	126	7 (5.5)	1 (0.8)	-	10 (7.9)	15 (11.9)	21 (16.7)	-	-	-	1 (0.8)	68 (54.0)	2 (0.15)	-	1 (0.8)
Pigeons° (Wild)	151	68 (45.0)	5 (3.3)	-	6 (4.0)	5 (3.3)	55 (36.4)	-	1 (0.7)	-	-	-	-	1 (0.7)	10 (6.6)
Dogs	257	18 (7.0)	2 (0.8)	6 (2.3)	59 (22.9)	4 (1.6)	37 (14.4)	61 (23.7)	50 (19.4)	-	11 (4.3)	2 (0.8)	4 (1.6)	1 (0.4)	2 (0.8)
Cats	204	5 (2.45)	7 (3.4)	-	6 (2.9)	1 (0.5)	59 (29.0)	36 (17.6)	-	-	-	-	2 (1.0)	79 (38.7)	9 (4.4)
Chickens	186	36 (19.3)	1 (0.5)	2 (1.1)	54 (29.0)	18 (9.7)	12 (6.45)	3 (1.6)	-	-	8 (4.3)	16 (8.6)	2 (1.1)	13 (7.0)	21 (11.3)
Pigs	138	5 (3.6)	1 (8.0)	-	27 (19.6)	1 (0.7)	42 (30.4)	3 (2.2)	-	-	1 (0.7)	21 (15.2)	-	12 (8.7)	15 (10.9)
Cows	201	2 (1.0)	-	13 (6.5)	7 (3.5)	-	10 (5.0)	71 (35.3)	3 (1.5)	-	-	51 (25.4)	3 (1.5)	14 (7.0)	27 (13.4)
Muskrats	145	67 (46.2)	11 (7.6)	4 (2.7)	-	37 (25.5)	-	7 (4.8)	-	-	2 (1.4)	12 (8.3)	2 (1.4)	2 (1.4)	1 (0.7)
Racoons	125	26 (21.0)	4 (3.2)	1 (0.8)	45 (36.0)	6 (4.8)	3 (2.4)	20 (1.6)	7 (5.6)	-	-	-	3 (2.4)	10 (8.0)	-
Horses°	87	3 (3.4)	-	1 (1.1)	16 (18.4)	10 (11.5)	35 (40.2)	-	-	20 (23.0)	-	-	-	2 (2.3)	-
Turkeys°	53	48 (90.6)	-	-	-	-	1 (1.9)	-	-	-	-	-	2 (3.8)	-	2 (3.8)

* The F.S. grouping is comprised of fecal streptococci which were non-indentifiable by biochemical methods but could be identified serologically by their group D antigen.

\+ The Non F.S. grouping is comprised of Gram (+), Catalase (-), Non-Group D.

° 1985 Data.

Percentages in Parenthesis

Sources of Faecal Pollution during Wet Weather Surveys

The evaluation of the proportional recoveries of E. coli and enterococci demonstrates a greater relative impact on indicator bacterial recoveries from non-faecal sources during wet weather events than during dry weather. This is not surprising since there would be inputs into the Humber River/Black Creek area of large volumes of storm water from sewers and direct runoff. Despite the fact that faecal bacterial inputs may be proportionally lower than total indicator bacterial inputs during wet weather, it was evident from the actual concentrations of E. coli and enterococci that total loadings were increased. These increased loadings would come from human sources (combined sewers, illegal connections to storm sewers, etc.) and from non-human sources including a number of different types of animals and birds whose faeces can be directly deposited or carried by storm water. The changing nature of the faecal inputs is reflected by changes in the proportional representation of the different streptococcal species.

Some other factors that could effect the level of faecal pollution are the duration of the wet event, its intensity, surface water temperatures during the event, and how recently a previous storm had occurred. The October event (Wet Survey 1) was of short duration. Only one discharge peak was observed during this event and temperatures were higher than during the November event (Wet Survey 2) which occurred over a longer period of time and had two discharge peaks. Therefore, differences in the bacterial populations would be expected between the two wet weather events.

October Event (Wet Survey 1)

The proportional changes noted in the streptococcal populations during this wet weather event in comparison to the dry weather results (i.e. increased S. faecalis var. liquefaciens, decreased S. faecium) when compared to the faecal data (Table 16) are suggestive of a decrease in human faecal pollution relative to non-human inputs. If the data obtained at the University of Toronto (Table 16) is representative of human faecal inputs in this area, an increase in human faecal wastes in comparison to other waste discharges would have resulted in the percent

recovery of S. faecium increasing to a greater degree than S. faecalis. This does not, however, mean that there are lower human faecal loadings to the system. Increases in the actual concentration of organisms such as S. faecium may result in part from increases in human faecal inputs particularly in areas where other potential indicators of sanitary sewage such as Pseudomonas aeruginosa (Tabel 3.6, Humber River Bacteriological Study. Gore and Storie, 1984), are seen to increase.

A major difficulty in interpreting the data from any given station is that it is being effected by a large number of inputs upstream and the greater increases in bacterial inputs from non-human sources mask the input from human sources. Distances travelled between input and detection will also effect the results due to changes in population distribution that occur because of bacterial die-off and sedimentation/resuspension.

The increased proportion of S. faecalis var. liquefaciens during this first wet event could be a combination of greater inputs from birds such as geese and pigeons, wild animals such as muskrats (Table 16) and non-faecal sources such as plants. Added to these sources will also be recent human faecal inputs.

Streptococcus faecium var casseliflavus has been recovered from plants (Mundt and Graham 1968) which may account for part of the increase in this organism in surface water bodies during wet weather events (Table 13). If this "non-faecal" streptococcal variety survives better in the natural environment, it may also be found in resuspended sediments caused by increased flows. Fresh inputs from non-human faecal sources such as geese and muskrats can also increase S. faecium var. casseliflavus concentrations. The highest percent recoveries of this variety were obtained at station H3; an area frequented by geese. The different relative recoveries of S. faecium var. casseliflavus on a m-ME and m-Enterococcus agar (Table 11, 13) is undoubtably related to the method differences as discussed earlier.

Streptococcus faecalis var. zymogenes can be isolated from non-faecal sources (Moussa 1965), however, the organism was also found in some faeces (Table 16) partilcularly cows, dogs and gulls. Streptococcus bovis and the S. bovis variant are found in the faeces of cows, dogs, gulls, cats as well as racoons. Both these organisms were recovered from the Humber River at the downstream locations

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during this wet event. Since S. bovis does not survive well in surface waters, it is probable that its source in the lower Humber sampling area (B1, H2 and H1) is from dog, gull, cat and racoon faeces rather than from upstream pasture land runoff.

Streptococcus avium was first found in the faeces of birds, although it can be isolated from other animals such as dogs (Mundt 1982). Its presence in the survey area may be due to birds such as pigeons and gulls, but some undoubtably come from other sources. S. durans is recovered from all faeces but is particularly high in wild pigeons thus increases in S. durans in a downstream direction could in part be due to major bird inputs. However, inputs from humans and other animals such as dogs should not be ruled out and the survival of this species in surface waters could influence its increasing recovery.

November Event (Wet Survey 2)

The longer duration, colder temperatures and higher discharge of the November storm event could result in impacts on a given point from further upstream or greater dilution of recent inputs. This in turn might result in proportionally lower faecal contamination as exhibited by the lower indicator bacterial concentrations on all the media (except enterococci on m-ME).

The faecal material present during this storm event appears to be more recent in nature then the first event. It has, however, been diluted and the large number of non-faecal inputs present between the stations masks the effect of the different types of faecal inputs (i.e. human and non-human).

The increased representation of non-enterococcal faecal streptococci at stations H3 and H2 on m-Ent agar suggests a greater upstream input from animal sources such as birds (e.g. pigeons) and dogs. It is possible that in addition to inputs of dog faeces increases in S. bovis may be partly due to cattle in the upstream areas. The decreased temperature and higher flow conditions of this event might allow for longer survival (time and distance) of these organisms, but this would require further assessment to be substantiated. The increases in S. durans could be the result of non-human inputs (e.g. pigeons and dogs) particularly at H3 while increases in S. faecalis var. liquefaciens probably arise from a wide variety of sources.

A lower representation of S. faecium var. casseliflavus on m-Ent could indicate lower inputs from sources such as geese and muskrats. Nonetheless, even though there was a decreased representation of this organism in a downstream direction, its overall recovery increased over the Wet Survey 1 levels and could indicate increased transport of contaminated resuspended sediments, or increased input from environmental sources due to land runoff. A reverse trend in the S. faecium var. casseliflavus population representation was observed on m-ME and relative concentrations also increased in the downstream direction suggesting an increased effect of resuspended contaminated sediments or more recent non-human faecal inputs at station B1 and particularly H2.

Small increases in S. faecium could in part be due to human faecal inputs, but the streptococcus population distribution in human faeces is somewhat diverse, due to factors such as diet, so it is not possible to make any definitive conclusions. In fact, at station B1, which is located below combined sewer outflows (an area where human faecal input is to be expected during wet weather conditions), the S. faecium representation was lower than during the other surveys and S. faecalis var. faecalis levels were increased. Since this organism can also be isolated from human faeces, (Mead 1972) its presence could still be an indication of human faecal input.

The streptococcal population patterns exhibited on m-ME agar suggest different impacts which, as discussed earlier, may represent the difference between more recent inputs with some impact from environmentally acclimatized bacteria (possibly those that survive in the sediments) that would grow on the restrictive m-ME and inputs from further upstream. These upstream inputs may consist of environmentally stressed bacteria and would thus be more likely to grow on the less harsh m-Ent agar.

The tendency for lower representative populations of S. avium, S. bovis and S. durans on m-ME are suggestive of a lower relative representation of non-human faecal material, or, more likely, less recent input from these sources. The fact that overall these organisms are more readily recovered from the upstream stations (B2, H3) indicates that there may be inputs from non-human faecal sources in the upper Humber region (i.e., pasture land runoff) impacting on the upstream stations in the

survey area due to increased flow and the long duration of the event.

The proportion of S. faecium recovered on m-ME is again higher than that recovered on m-Ent agar at all stations except H2 and also higher than during the first survey (again with the exception of station H2). The increase is most evident at station B1. This difference in recovery on m-ME may indicate increases in human inputs at both the Black Creek stations and at station H1 and human or possibly bird inputs at station H3 with less recent inputs impacting on station H2. However, as previously mentioned, this is still speculative because the medium has not been tested for its ability to detect recent faecal input.

It should be noted that the proportional population level of S. faecalis var. faecalis is lower at all stations in the Humber during this second event. Since this organism is mainly found in the faeces of humans and animals (Sherman 1937, Mead 1972), this may indicate an overall decrease in faecal loading, during this event, relative to non-faecal inputs.

The results at station H2 suggest that problems with interpretation of bacterial levels can occur if there is a major impact of non-faecal or environmentally stable streptococcal species present, possibly as a result of sediment transport. Both S. faecium var. casseliflavus and S. faecalis var. liquefaciens have been recovered from non-faecal sources which may account for their increased levels at the downstream stations. If this is the case, then what appears to be less recent faecal inputs at station H2 may in fact be the result of a masking effect caused by increases in the environmental isolates.

Conclusions

1) The specificty of m-TEC-IG and m-ME agars was acceptable under both dry and wet weather survey conditions in surface waters impacted by a variety of sources. Some specific conditions, such as determination of faecal contamination by pulp and paper mill effluents using m-TEC-IG, will still have to be ascertained. Caution must be used in the interpretation of m-ME data, particularly during wet weather surveys. The increased levels obtained using this medium may in part be related to "non-faecal" inputs and/or sediment resuspension.

2) The conclusions reached about levels of faecal comtamination from the 1983 Humber River/Black Creek survey (Gore and Storie) were supported by this study. The fact that E. coli made up a large portion of the faecal coliform population was proof of recent faecal contamination. The relatively high recoveries of enterococci supported the E. coli results. Both sets of data provide evidence for an increase in recent faecal loadings in a downstream direction as well as during wet weather events. However, during wet weather, the proportion of faecal indicator bacteria from faeces decreased, possibly as a result of proportionally greater increases form non-faecal sources (i.e. Klebsiella spp., Enterobacter spp., and Citrobacter spp. can all be found in non-faecal environments). The faecal loadings exhibited during the second event, although lower than the first, appear to be from more recent inputs.

3) Interpretation of bacterial population distributions should not be considered absolute since: a)Bacterial populations in faeces from human and non-human sources, although showing some differences in distribution, also have a number of areas of overlap. This would indicate that a study designed to minimize the number of potential inputs is warranted; b) The original water quality survey design relied on too few sampling stations (6 dry and 4 wet) for the size of the area surveyed, thus allowing potential impacts from a large variety of inputs between the stations.

4) The streptococcal species distribution during dry weather suggested faecal inputs from a wide variety of sources. There was a strong indication of inputs of human faeces into the survey area with the possibility of increased faecal loadings

in a downstream direction. Human faecal inputs appeared to be having a greater impact on the Black Creek survey area than in the Humber River. It was also apparent that major contributions come from non-human sources such as geese, dogs and pigeons.

5) The streptococcal population data indicated that total human faecal inputs may be higher during wet weather compared to dry weather conditions; however, their impact was masked by an increase in non-human faecal and non-faecal, inputs as well as less recent inputs caused by factors such as sediment resuspension. The data collected from the second wet event suggested that human faeces formed a greater portion of the total faecal input than during the first wet weather event, particularly in Black Creek.

Recommendations

1) Future attempts to use bacterial population distributions should be concentrated in small survey areas preferably gathering data from upstream, at, and downstream of an input source.

2) Studies should be carried out to assess bacterial populations in sanitary and true storm sewage since this is how much of the faecal loading, particularly human, enters surface waters.

3) More information is needed concerning the impact of sediments on bacterial concentration and population distribution in the water column. Similarly an investigation of the bacterial populations in soil and on vegetation could be of value.

4) More data on bacterial populations in the faeces of wild animals and birds would be useful. It may also be necessary to distinguish the bacterial species in a wide variety of human faeces, as well as in raw sewage.

5) The effect of fresh faecal inputs on the relative recoveries from m-ME medium in comparison with m-Enterococcus agar is requried to determine if there is any significant pattern that could be used to indicate if the pollution was of a recent nature.

6) More information on the relationship betweeen the presence of P. aeruginosa and point source inputs, such as sanitary sewage, would provide valuable corroborative data for studies such as this. Serotyping and antibiotic resistance testing could also provide valuable additional data when attempting to trace and identify sources.

7) The relative die-off rates of the different streptococcal species and varieties would provide data to assess the potential site (distance) of impact from a given source. Information on changes in faecal coliform to faecal streptococcus ratios downstream from known sources would assist in determining the identity of unknown sources.

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APPENDIX A: Media Formulations

Appendix A - 1

m-TEC Agar (Dufour, 1975)

The M-TEC medium is formulated for the quantitative recovery of thermotolerant faecal coliform bacteria: Escherichia coli, Klebsiella spp., Enterobacter spp. and Citrobacter spp. from water and waste water analysis by membrance filtration methods. It selects for Gram negative non-spore forming rod-shaped bacteria that can ferment lactose within 24 hours at an elevated temperature (44.5°C) on the basis that bacteria possessing these characteristics inhabit the gastrointestinal tract of humans and other animals. The medium is incubated at 35°C for 2 hours as a resuscitation step then for 21 hours at 44.5± o.5°C, making a total incubation period of 23± 1 hours. Target counts of all yellow, yellow-green and yellow-brown colonies are recorded as the faecal coliform count.

A second step can be incorporated into the recovery method to differentiate Escherichia coli from other thermotolerant faecal coliform bacteria (members of the latter group can come from sources other than faeces). The second step involves removing the sample filter from the m-TEC medium and placing it onto a filter pad saturated with a urea solution containing phenol Red (Dufour, 1981). The filter remains in contact with the urea for 15 minutes to allow deamination of the urea by non-E. coli coliform bacteria. Then, a second count of all the urease negative (yellow) colonies is made and recorded as the E. coli count.

m-TEC Medium Formulation	**per Litre**
Proteose Peptone #3	5.0 g
yeast Extract	3.0 g
Lactose	10.0 g
NaCl	7.5 g
K_2HPO_4	3.3 g
KH_2PO_4	1.0 g
Sodium Lauryl Sulphate	0.2 g
Sodium Desoxycholate	0.1 g
Bromocresol Purple	0.08 g
Bromo Phenol Red	0.08 g
Agar	15.0 g
Distilled H_2O	1000 mls

Appendix A - 2

m-Enterococcus Agar (Slanetz and Bartley A 1957)

m-Enerococcus agar is formulated for the quantitative recovery of faecal streptococci: Streptococcus faecalis var., S. faecium var., S. durans, S. bovis, S. equinus and S. avium from water and waste water analysis by membrane filtration methods. The medium contains sodium azide to inhibit the growth of Gram negative organisms and triphenyltetrazolium chloride which is reduced by the faecal streptococci to a pink to maroon colour (depending on the degree of reduction). The medium is incubated for 48 hours at 35°C and a target count of all pink to maroon colonies is made.

m-ENT Medium Formulation	**per Litre**	
Bacto-Tryptose	20	g
Bacto-Yeast Extract	5	g
Bacto-Dextrose	2	g
Dipotassium phosphate	4	g
Sodium Azide	0.4	g
Bacto-Agar	10	g
2,3,5 - Triphenyl Tetrazolium Cl	0.1	g
Distilled H_2O	1000	ml

(Do not Autoclave)

Appendix A - 3

m-TEC Agar with Indoxyl-β-D-glucoside (IG) (Dufour, 1979)

m-TEC-IG is formulated for the quantitative recovery of Escherichia coli from water and waste water analysis using membrane filtration methods. Its formulation is similar to regular m-TEC agar and it selects for thermotolerant faecal coliform bacteria able to ferment lactose within 24 hours at an elevated temperature (44.5°C). In addition, the medium contains IG which allows for a single step differentiation of E. coli from other thermotolerant faecal coliform bacteria. Non-E. coli faecal coliforms possess an enzyme which breaks down the IG to produce a blue halo on the filter surrounding the bacterial colony. The medium is incubated in an identical fashion to m-TEC and target counts of all yellow, non-blue haloed colonies are recorded as the E. coli count.

m-TEC Medium Formulation	**per Litre**
Proteose Peptone #3	5.0 g
Yeast Extract	3.0 g
Lactose	10.0 g
NaCl	7.5 g
K_2HPO_4	3.3 g
KH_2PO_4	1.0 g
Sodium Lauryl Sulphate	0.2 g
Sodium Desoxycholate	0.1 g
Bromocresol Purple	0.08 g
Bromo Phenol Red	0.08 g
Agar	15.0 g
Distilled H_2O	1000 mls

After Autoclaving and cooling to 50°C add
Indoxyl-°-D-Glucoside 0.25 in 5 ml ETOH

Appendix A - 4

m-ME Agar (with Indoxyl-β-D-Glucoside) (Dufour, 1980)

m-ME agar is formulated for the quantitative recovery of enterococci: Streptococcus faecalis, S. Faecalis var. liquefaciens, S. faecalis var. zymogenes, S. faecium, S. faecium var. casseliflavus and S. durans from water and wastewater analysis using membrane filtration methods. The medium contains both sodium azide and triphenyltetrazolium chloride like M-Enterococcus agar. It also contains 2 antibiotics to further decrease background organisms. The addition of indoxyl-b-D-glucoside (IG) allows for differentiation of enterococci from other faecal streptococci which will grow on the medium. The enterococci group possess an enzyme which breaks down (IG) producing a blue halo on the membrane filter surrounding the colony. The medium is incubated for 24 hours at 41.5°C and a target count of all blue haloed colonies is made.

m-ME Medium Formulation

Peptone	10.0 g
Yeast Extract	30.0 g
Sodium Chloride	15.0 g
Sodium Azide	0.15 g
Actidione	0.05 g
Agar	15.0 g
distilled H_2O	1000 mls
(After autoclaving) Naladixic acid	240 mg in 3 mls sterile DH_2O and 0.2 ml 10 N NaOH
Triphenyltetrazolium Chloride	20 mg
Indoxyl-β-D-glucoside	750 mg in 5 mls ETOH (95%) and 5 mls DH_2O

APPENDIX B: Identification of faecal streptococci

Appendix B - 1

Relationships of Enterococci, Group D Streptococci and Faecal Streptococci

FECAL STREPTOCOCCI

Enterococci

- S. faecalis
- S. faecalis var liquefaciens
- S. faecalis var zymogenes
- S. faecium
- S. faecium var casseliflavus
- S. durans

Other Group D Streptococci

- S. bovis
- S. equinus
- S. avium (GRP D, Q)

Viridans Group

- S. mitis
- S. Salivarius

ref. Hartman et al, Indicator Organisms - A Review Taxonomy of the Faecal Streptococci. Int., Journ. Syst. Bact. V16-2, Apr 1966, pp 197-221.

Appendix B - 2

Identification Scheme for Faecal Streptococci

1) Pick isolate colonies from m-Enterococcus or other similar agar.

2) Transfer the growth to blood agar (5% rabbit's blood) and streak out for isolated colonies. Incubate the blood plates at 35°C for 24 hours.

3) Examine incubated blood plates for purity and growth of the culture. Select one isolated colony from pure cultures only and prepare a reservoir of growth on Brain-Heart Infusion agar (BHI). Incubate the BHI plates at 35°C for 24 hours.

4) After incubation, use reservoir of growth on BHI agar to check the Gram reaction (3% KOH method) and catalase reaction of the isolate.

5) If the isolate is Gram positive, catalase negative, then use the growth on BHI agar to inoculate the following physiological tests:

1. Bile Esculin Agar
2. Todd Hewitt Broth for growth at 10°C
3. Todd Hewitt Broth for growth at 45°C
4. 6.5% NaCl (in Heart Infusion Broth)
5. Thornleys Arginine dihydrolase medium
6. 2% soluble Starch (in blood agar base)
7. Tellurite Agar (0.04% Potassium Tellurite
8. 1% Arabinose (in heart infusion broth)
9. Galatin (12% in heart infusion broth)
10. Pyruvate Broth
11. 1% Mannitol (in heart infusion broth)
12. 1% Lactose (in heart infusion broth)
13. 1% Sorbose (in heart infusion broth)
14. Methylene Blue Milk (0.1% Methylene blue in Skim Milk Broth)

The above series of tests will allow for separation of enterococcus species from other faecal streptococci and from non-faecal streptococci. (See Table #1) Potential S. bovis, S. equinus and S. avium isolates must be tested seriologically for the group D Antigen (See Serological Testing Method) before their identification can be confirmed, since they show biochemical reactions similar to the viridans streptococci group. Variants of S. faecalis must be tested for their haemolysis reactions under anaerobic conditions on 5% rabbit's blood Agar.

TABLE 1: Some Physiological Reactions of the Faecal Streptococci and Some Physiologically Similar Viridans Streptococci and Aerococci Useful for Differentiation

	Bile esculin	Growth 10°C	Growth 45°C	Growth 6.5% NaCl	Arginine	Starch	Tellurite	Arabinose	Gelatin	Tetrazolium	Heamolysis	Yellow pigment	Methylene blue milk	Pyruvate	Mannitol	Lactose	Sorbose	Serogroup
S. faecalis	+	+	+	+	+	-/+	+	-	-	+	OL/γ	-	+	+	+	+	-	D
S. faecalis v. liquifaciens	+	+	+	+	+	-	+	-	+	+	OL/γ	-	+	+	+	+	-	D
S. faecalis v. zymogenes	+	+	+	+	+	-	+	-	V	+	β	-	+	+	+	+	-	D
S. faecium	+	+	+	+	+	-	-	+	-	-		-	+	-	+	+	-	D
S. faecium v. casseliflavus	+	+	+	+	+	-	V	+	-	V		+	+	-	+	+	-	D
S. durans	+	+	+	+	+	-	-	-	-	-		-	+	-	-	+	-	D
S. avium	+	+	+	+	-	-	-	+	-	V		-	-	+	+	+	+	Q/D
S. bovis	+	-	+	-	-	+	-	-	-	V		-	V	-	+	+	-	D
S. bovis variant	+	-	+	-	-	-	-	-	-	V		-	V	-	-	+	-	D
S. equinus	+	-	+	-	-	-	-	-	-	-		-	-	-	-	-	-	D
S. mutans	-/+	-	+/-	-	-	-									+	+		E/NG
S. MG intermedius	-/+	-	-/+	-	+/-	+/-									-	+		F/NG
S. salivarius	(-d)	-/+	+/-	-	-	-									-	+		K/NG
S. sanguis I	(-d)	-	+/-	-	+/-	+/-									-	+		F/NG
Aerococcus sp.	+/-	+	-	+	-													

Note: Some S. bovis (starch-ve) variants cannot be distinguished from certain viridans streptococci (i.e. S. MG intermedius and S. sanquis I) except by serology. The above reaction patterns arre the most common and variants for any particular test that may occur.

Streptococcus - Seriological Grouping

Principle of Test:

The majority of streptococcus species possess group-specific antigens which are usually carbohydrate structural components of the cell wall. These antigens can be extracted in soluble form and identified by precipitation reactions with homologous antisera. There are a number of ways to extract these antigens from the cell wall including 1) Hot HCL extraction 2) Hot formamide extraction 3) Autoclave extraction 4) Sonication and 5) Enzyme extraction. Each extraction method has certain advantages and disadvantages. Generally the autoclave extraction method and the enzyme extraction method are simple yet reliable procedures for all groups including group D. Some Group D streptococcus species (i.e. S. bovis, S. equinus and S. avium), however, contain relatively small amounts of this antigen and these may require more severe extraction procedures (i.e. sonication).

The antigen-antisera precipitation reactions can be performed in various ways including 1) Capillary precipitin test 2) Slide agglutination reaction 3) Electrophoretic methods.

Probably the simplest method to employ is the slide agglutination procedure whereby group-specific antibody coated latex particles are reacted with the antigen extract.

There are commercially prepared kits available which provide the enzyme for an enzyme extraction, a reaction slide and antibody coated latex particles for various serogroups (generally group A,B,C,D,F and G). These latex particles can also be reacted with extract from any other extraction procedure.

Methods

Enzyme Extraction - latex agglutination (Commercially available kit)

1) Quality control - The kit contains a vial of polyvalent antisera. Mix one drop of each latex compound into one drop of antisera to ensure that each latex compound reacts appropriately
2) Rehydrate the lyophilized extraction enzyme
3) Cells for the extraction procedures for the unknowns can be taken from a plate or broth culture
   a) Plate - Sweep a light loopful of growth from a 24 hour blood plate and emulsify in 0.4 mL extraction enzyme

OR

   b) Broth - take one drop of a 24 hour brain heart infusion + 1% dextrose broth culture and add to 0.4 mL extraction enzyme
4) Incubate the extraction enzyme cell mixture one hour at 37°C (water bath)
5) Dispense one drop of the appropriate latex per corresponding circle on the agglutination slide
6) Add one drop of the incubated extract to each latex drop
7) Mix until smooth and milky using a separate applicator stick for each circle
8) Gently rock and rotate the slide until a reaction occurs in one of the circles (usually within one to two minutes)

   A positive agglutination is noted when the smooth milky emulsion changes to granular
9) Record which group antigen if any is present

Autocalve Extractions of Group Antigen

1) Grow pure isolate in Brain heart infusion broth plus 1% dextrose (final concentration) for 24 hr, 35°C, in 16 x 100 screw cap tubes, 8 mL broth per tube (tube must be suitable for centrifugation)
2) After incubation centrifuge broth culture at 3000 rpm, 10 min. to pack cells
3) Decant liquid into a disinfectant and retain cells
4) Add approximately 5 mL of aqueous 0.85% NaCl solution (physiological saline) to the tube and resuspend cells
5) Centrifuge the cells a second time at 3000 rpm, 10 min to pack cells
6) Decant liquid into a disinfectant and retain cells
7) Add 2-3 mL physiological saline to the tube and resuspend the cells
8) Autocalve the tube 15 min., 15 psi (121°C)
9) After autoclaving, allow to cool and centrifuge the tube at 3000 rpm, 5 min. to pack cells
10) Once the cells are packed the liquid (extract) phase can be used to perform the seriological testing. (See latex method.)

APPENDIX B-3

References used to develop Scheme for Faecal Streptococcus Identification

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